

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB05/000549

International filing date: 17 February 2005 (17.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB

Number: 0403452.6

Filing date: 17 February 2004 (17.02.2004)

Date of receipt at the International Bureau: 31 May 2005 (31.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



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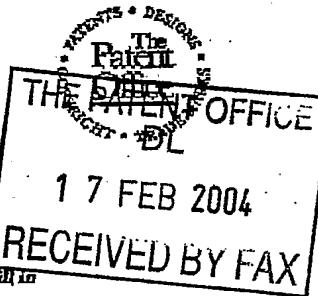


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The University of York
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7511405002

4. Title of the invention

Desaturase enzymes

5. Name of your agent (if you have one)

Harrison Goddard Foote

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DUPLICATE

Desaturase Enzymes

The invention relates to transgenic cells transformed with nucleic acid molecules which encode enzymes with desaturase activity and the use of these cells and enzymes in biocatalysis.

Desaturases are enzymes involved in the synthesis of long chain polyunsaturated fatty acids (PUFAs). PUFAs are fatty acids (FAs) which are essential to the normal functioning of a cell and their nutritional properties are well known. An example of a PUFA is docosahexanoic acid (DHA). DHA is a n-3 fatty acid that can be obtained directly from the diet or derived from metabolism of dietary linoleic and α -linolenic acid. The n-3 fatty acids are associated with health promoting properties. For example n-3 fatty acids have been described as anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and vasodilatory. As such, the role of DHA in the prevention and/or treatment of diseases such as coronary heart disease, hypertension, type II diabetes, ocular diseases, arthritis, cystic fibrosis and schizophrenia has been the focus of a great deal of medical research.

The production of PUFAs involves a consecutive series of desaturations and elongations of the fatty acyl chain to generate arachidonic acid (20:4 Δ 5,8,11,14) and docosahexaenoic acid (22:6 Δ 4,7,10,13,16,19). Several desaturases involved in this metabolic process have been isolated from marine microalgae, including *Phaeodactylum tricornutum* [5], *Euglena gracilis* [6] and *Pavlova lutheri* [7]. These membrane-bound desaturases are specific with respect to both chain length of the substrate and the double bond positions on the fatty acid. They belong to the class known as front-end fatty acid desaturases due to the fact that they introduce double bonds between the carboxy-group and pre-existing bond(s) of the fatty acid [1]. These desaturases contain a cytochrome *b5* domain at their N-terminus and three histidine motifs that are important for catalytic activity [10].

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Desaturase enzymes and the genes which encode them are known in the art. For example, WO03/064596 describes, amongst other things, transgenic cells transformed with omega 3 and delta 12 desaturase nucleic acid molecules and the use of these cells in the production of fatty acids. In particular the use of the omega 3 desaturase in the conversion of arachidonic acid to eicosapentaenoic acid and the use of the delta 12 desaturase in the conversion of oleic acid to linoleic acid. WO03/099216 also describes fungal desaturases and in particular transgenic plants modified to express fungal delta 15 desaturase enzymes.

5 Furthermore, US2003/0157144 and US2003/0167525 disclose delta 5 and delta 6 desaturase genes in the conversion of dihomoylinolenic acid to arachidonic acid and linoleic acid to γ -linolenic acid respectively. Moreover, US2003/134400 discloses delta 4 desaturase genes which are involved in the conversion of adrenic acid to ω 6-docosapentaenoic acid and in the conversion of ω 3- docosapentaenoic acid to docosahexaenoic acid. These rare fatty acids are used in pharmaceutical and cosmetic compositions and can be essential nutritional fatty acids.

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Besides the common FAs 16:0, 16:1 Δ 9, 18:0 and 18:1 Δ 9 found in most living organisms, trace amounts of more unusual fatty acids can be found in a wide range of species. For instance, presence of 16:1 Δ 11 has been reported in several species of *Pavlova*, in the Eustigmatophyte *Nannochloropsis oculata*, and in the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* [11,12,13]. This FA accounted for a very small portion of the total FAs in these microalgae, and its specific role in the algal cells is unknown. However, this FA is a very important precursor in the synthesis of sex pheromones in insects. Sex pheromones are species-specific blends of unsaturated fatty acid (UFA) derivatives that differ in terminal functional group and in the number, position and configuration (Z or E) of the double bond(s), which are produced by various acyl-CoA desaturases [14,15]. Simple monoene Δ 11 UFAs are the most prevalent precursors in the formation of major sex pheromone components in the modern *Lepidoptera* [16,17]. For instance, in the corn earworm *Helicoverpa zea*, which produces a pheromone mixture of Z11-16:Ald and

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Z9-16:Ald in a 30:1 ratio, the most abundant desaturase-encoding transcript is *HzeALPAQ* (also called *HzPGDsl*) which encodes a Δ11-desaturase that does not possess a cytochrome *b5* extension, and therefore requires free cytochrome *b5* for activity. Many acyl-CoA Δ11-desaturases with different specificities have been 5 isolated from insects [14,15], but none from other species. We describe the first characterisation of a cytochrome *b5* desaturase exhibiting Δ11-desaturase activity.

According to an aspect of the invention there is provided a transgenic cell comprising a nucleic acid molecule which comprises a nucleic acid sequence which nucleic acid 10 molecule consists of the sequences as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b, or nucleic acid molecules which hybridise to these sequences, wherein said nucleic acid molecules encode a polypeptide which has desaturase activity.

15 In a preferred embodiment of the invention said hybridisation conditions are stringent hybridisation conditions.

In a preferred embodiment of the invention said nucleic acid molecule comprises a nucleic acid sequence which has at least 30% homology to the nucleic acid sequence 20 represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b. Preferably said homology is at least 40%, 50%, 60%, 70%, 80%, 90%, or at least 99% identity with the nucleic acid sequence represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b and which encodes a polypeptide which has desaturase activity.

25 In a preferred embodiment of the invention said nucleic acid molecule comprises the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b. Preferably said nucleic acid molecule consists of the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b.

30 In a further preferred embodiment of the invention said cell over-expresses said desaturase encoded by said nucleic acid molecule.

In a preferred embodiment of the invention said over-expression is at least 2-fold higher when compared to a non-transformed reference cell of the same species.

5 Preferably said over-expression is: at least 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or at least 10-fold when compared to a non-transformed reference cell of the same species.

In a preferred embodiment of the invention said nucleic acid molecule is a cDNA.

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In yet a further preferred embodiment of the invention said nucleic acid molecule is a genomic DNA.

15 In a preferred embodiment of the invention said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 10a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 11$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 10a and encodes a polypeptide with $\Delta 11$ -desaturase activity.

20 In an alternative preferred embodiment of the invention said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 8a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 6$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 8a and encodes a polypeptide with $\Delta 6$ -desaturase activity.

25 In a preferred embodiment of the invention said transgenic cell is a eukaryotic cell.

In an alternative preferred embodiment of the invention said cell is a prokaryotic cell.

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In a further preferred embodiment of the invention said eukaryotic cell is a plant cell.

Plants which include a plant cell according to the invention are also provided as are seeds produced by said plants.

5 In a preferred embodiment of the invention said plant is selected from: corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), flax (*Linum usitatissimum*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*),
10 peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Iopmoea batatas*), cassava (*Manihot esculenta*), coffee (*Cofea spp.*), coconut (*Cocos nucifera*), pineapple (*Anana comosus*), citrus tree (*Citrus spp.*) cocoa (*Theobroma cacao*), tea (*Camellia senensis*), banana (*Musa spp.*), avacado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifer indica*), olive (*Olea europaea*),
15 papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia intergrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), oats, barley, vegetables and ornamentals.

20 Preferably, plants of the present invention are crop plants (for example, cereals and pulses, maize, wheat, potatoes, tapioca, rice, sorghum, millet, cassava, barley, pea), and other root, tuber or seed crops. Important seed crops are oil-seed rape, sugar beet, maize, sunflower, soybean, sorghum, and flax (linseed). Horticultural plants to which the present invention may be applied may include lettuce, endive, and vegetable brassicas including cabbage, broccoli, and cauliflower. The present
25 invention may be applied in tobacco, cucurbits, carrot, strawberry, sunflower, tomato, pepper.

30 Grain plants that provide seeds of interest include oil-seed plants and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and

peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

According to a further aspect of the invention there is provided a seed comprising a 5 plant cell according to the invention. Preferably said seed is from an oil seed plant.

According to a yet further aspect of the invention there is provided a reaction vessel comprising at least one polypeptide according to the invention, fatty acid substrates and co-factors wherein said vessel is adapted for the desaturation of said fatty acids 10 substrates.

In a preferred embodiment of the invention said polypeptide is expressed by a cell according to the invention.

15 Preferably said cell is a eukaryotic cell, for example a yeast cell.

In an alternative preferred embodiment of the invention said cell is a prokaryotic cell.

According to a further aspect of the invention there is provided a method to 20 desaturate a fatty acid substrate comprising the steps of:

- i) providing a reaction vessel according to the invention; and
- ii) growing said cells contained in said reaction vessel under conditions which allow the desaturation of at least one fatty acid substrate.

25 An embodiment of the invention will now be described by example only and with reference to the following tables and figures:

Table 1 illustrates the composition of major fatty acids in *T. pseudonana*;

30 Table 2 illustrates the major fatty acids of pYES and pYDESN yeast transformants with and without addition of exogenous saturated fatty acids;

Table 3 illustrates the $\Delta 6$ desaturase activity of TpDESI compared to that of an homologous *Phaeodactylum tricornutum* desaturase;

5 Figure 1 illustrates the predicted protein sequences with homology to front-end desaturases derived from the *T. pseudonana* draft genome. Sequence alignments of 12 putative *T. pseudonana* desaturases with other functionally characterised front-end desaturase enzymes identified three main blocks of homology that represent the functional domains of front-end acyl desaturases (A). The darker shaded box
10 highlights the cytochrome *b5* haem-binding domain and shaded boxes indicate three histidine boxes. See Material and Methods for Genbank accession number and source species of the functionally characterised enzymes. A phylogenetic tree of nine *T. pseudonana* desaturases with other enzymes was constructed (B). By removing the regions containing gaps (ambiguous alignment region), a dataset was created from an
15 alignment originally made with clustalX. The tree was constructed from the dataset using Phylip3.5c software package and bootstrap analyses were carried out with 1000 replicates. Only well supported nodes (over 70%) are indicated with bootstrap values. All branches are drawn to scale as indicated by the scale bar (=0.1 substitutions/site).
20 TpDESN sequence is 477 amino acids long (C). The cytochrome *b5* haem-binding domain is on a shaded background and the three histidine-boxes are framed;

Figure 2 illustrates RT-PCR expression analysis of *TpdesN*. Cells were harvested at different stages of growth for total RNA extraction and cDNA synthesis (A). PCR was performed on cDNA derived from reverse transcribed RNA using *TpdesN* and 25 *18s rRNA* specific primer pairs (B). PCR was carried out on undiluted (lane 1) and five-fold serial dilutions (lane 2-4) of each cDNA. The 18S rRNA gene was used as a control of cDNA synthesis. EE: early exponential phase, LE: late exponential phase, ES: early stationary phase;

30 Figure 3 illustrates GC analysis of FAMEs from yeast transformed with the empty plasmid pYES2 or the plasmid containing TpDESN. Invsc1 yeast strain transformed

with either pYES2 (A) or pYDESN (B) were induced for three days at 20°C without supplementation before sampling for fatty acid analysis. I. S. internal standard (17:0). The experiment was repeated three times and results of a representative experiment are shown;

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Figure 4 illustrates mass spectra of DMDS FAME adducts from pYDESN transformed yeast. Mass spectrum of the DMDS adduct of 16:1Δ9 FAME, present in all yeast samples (A). Mass spectrum of the DMDS adduct of 16:1Δ11 FAME, which was only found in yeast transformed with pYDESN (B). Picolinyl esters with spectra characteristic of 16:1Δ11 were also identified in these samples (data not shown); and

10 Figure 5a is the genomic nucleic acid sequence of the desaturase A from *T. pseudonana*; Figure 5b is the cDNA sequence desaturase A; Figure 5c amino acid sequence;

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Figure 6a is the genomic nucleic acid sequence of desaturase B from *Thalassiosira pseudonana*; Figure 6b is the partial amino acid sequence;

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Figure 7a is the nucleic acid sequence of desaturase E from *Thalassiosira pseudonana*; Figure 7b is the amino acid sequence;

Figure 8a is the nucleic acid sequence of desaturase I from *Thalassiosira pseudonana*; Figure 8b is the cDNA sequence; and Figure 8c is the amino acid sequence;

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Figure 9a is the nucleic acid sequence of desaturase K from *Thalassiosira pseudonana*; Figure 9b is the amino acid sequence;

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Figure 10a is the nucleic acid sequence of desaturase N from *Thalassiosira pseudonana*;

Figure 11a is the nucleic acid sequence of desaturase O from *Thalassiosira pseudonana*; Figure 11b is the cDNA sequence; and Figure 11c is the amino acid sequence;

5 Figure 12A and 12B is a GC analysis of FAMEs from yeast expressing TpDES1 with exogenous substrates 18:2Δ9,12 (A) and 18:3Δ9,12,15 (B). New FAs produced from endogenous and exogenous substrates are underlined.

Figure 13 is an illustration of fatty acid synthesis pathways.

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Materials and Methods

15 Identification of putative *Thalassiosira pseudonana* desaturase-coding sequences and phylogenetic analysis with other functionally characterised desaturases

The draft genome of the diatom *T. pseudonana* has been sequenced to approximately nine times coverage by the whole genome shotgun method. Sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/) and the raw sequence data were downloaded and installed on a local server. Batch tblastn searches were carried out using protein sequences of the following 13 known desaturases as query, including PIDES1 (AY332747, *Pavlova lutheri*), TEAD4 (AF489589, *Thraustochytrium sp.* ATCC 21685), TFADS (AF489588, *Thraustochytrium sp.* ATCC 21685), PtDEL5 (AY082392, *Phaeodactylum tricornutum*), PtDEL6 (AY082393, *Phaeodactylum tricornutum*), EgDEL8 (AF139720, *Euglena gracilis*), EgDEL4 (AY278558, *Euglena gracilis*), ZfDEL (AF309556, *Danio rerio*), BoDEL6 (U79010, *Borago officinalis*), HsDEL5 (AF084558, *Homo sapiens*), HsDEL6 (AF084559, *Homo sapiens*), CeDEL6 (AF031477, *Caenorhabditis elegans*) and CeDEL5 (AF078796, *Caenorhabditis elegans*).

All non-redundant sequences with an E value less than 0.001 were retrieved and assembled into contigs using the CAP3 sequence assembly program [18]. The contigs were translated into amino sequences in three frames in the orientation indicated by tblastn result. Putative desaturase gene models were constructed manually based on sequence homology and in frame GT-AG intron boundaries were identified.

Deduced amino acid sequences of all 12 putative desaturase sequences of *T. pseudonana* were aligned with the above 13 functionally characterised desaturases from other species, using ClustalX version 1.8 [19]. The alignment was then 10 reconciled and further adjusted. Only nine near full-length *Thalassiosira* sequences were retained for further analyses.

A dataset of 250 conserved residue positions was used for construction of the phylogenetic tree. Distance analysis used the program protdist of the Phylip 3.5c package with a PAM250 substitution matrix and a tree was then built from the matrix using fitch (Fitch-Margoliash method). Bootstrap analyses were carried out with 1500 replicates using the neighbour-joining algorithm.

Cultivation of *T. pseudonana*

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T. pseudonana (CCAP 1085/12) was obtained from the Culture Collection of Algae and Protozoa (Dunstaffnage Marine Lab., Oban, PA34 4AD, Scotland, U.K.). The growth medium used was enriched artificial seawater medium (EASW), made up in 20 l batches as described previously [4]. The cultures were grown in one litre flasks 25 at 15°C with 50 μ E m⁻² s⁻¹ constant illumination, and aeration provided by shaking the flasks at 150 rpm.

Cell density was monitored by counting cells with a haemocytometer. Nitrate concentration was determined periodically during the culture time by measuring the 30 change of the medium absorbance at 220 nm [20].

RNA extraction, cDNA synthesis and RT-PCR analysis

5 Total RNA was extracted from frozen cells harvested at different stages of growth with an RNeasy plant mini kit (Qiagen). First strand cDNA was synthesised from three µg of DNase treated RNA using a Prostar First-strand RT-PCR kit (Stratagene). PCR was performed using undiluted and five-fold dilutions of cDNAs as followed: the reactions were heated to 95 °C for 5 min followed by 35 cycles at 95
10 °C for 30 s, 50 °C or 65 °C (for 18S rRNA and *TpdesN* respectively) for 30 s and 72 °C for 2 min, then a single 72 °C for 10 min. As a marker for constitutive expression, the 18S rRNA gene was amplified with the primer TH18S5' (5'-GGTAACGAATTGTTAG-3') and TH18S3' (5'-GTCGGCATAGTTTATG-3').
15 *TpdesN* cDNA was amplified using primers DESNR2 (5'-GTGAGAGCACTAACCAAGCTT-3') and DESN2 (5'-CAATCAGTAGGCTTCGTC G-3'). Aliquots of PCR reaction were electrophoresed through a 1% agarose gel. Identity of the diagnostic fragment amplified with *TpdesN* specific primers was verified by sequencing after cloning in the pGEM-T EasyVector (Promega).

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Functional characterisation of *TpDESI* in yeast

The entire *TpdesI* coding region was amplified from *T. pseudonana* cDNA with primers DesINB 5'- **GCGGGATCC**ACCATGGCTGGAAAAGGAGGAGAC-
25 - 3' (ORF start codon is indicated by bold type; underlined sequence is a *Bam*HI site; italic sequence is an added alanine codon, not present in the original sequence of *PidesI*) and DesICE 5'-**GCGAATTCTT**ACATGGCAGGGAAATC-3' (ORF stop codon is indicated in bold type; underlined sequence is a *Eco*RI site). The Expand High Fidelity PCR system (Roche) was employed to minimise potential PCR errors.
30 The amplified product was gel purified, restricted and cloned into the corresponding sites behind the galactose-inducible GAL1 promoter of pYES2 (Invitrogen) to yield

the plasmid pYDES1. This vector was transformed into *S. cerevisiae* strain Invsc1 (Invitrogen) by a lithium acetate method, and transformants were selected on minimal medium plates lacking uracil.

For functional expression, cultures were grown at 25°C in the presence of 2% (w/v) raffinose and 1% (w/v) Tergitol NP-40 (Sigma). Expression of the transgene was induced when OD_{600nm} reached 0.2-0.3 by supplementing galactose to 2% (w/v). At that time, the appropriate fatty acids were added to a final concentration of 50 μM. Incubation was carried out at 25°C for three days.

10 Functional characterisation of TpDES1 in yeast

Genomic DNA from *T. pseudonana* cells was extracted using the DNA isolation kit Puregene (Gentra Systems) and 100 ng was used to amplify the entire *TpdesN* coding region with primers *DesNNB* 5'-
15 **GC**GGGATCCACCATGGCGACTTCTCCGGC-3' (ORF start codon is indicated by bold type; underlined sequence is a *Bam*HI site; italic sequence is an added alanine codon, not present in the original sequence of *TpdesN*) and *DesNCE* 5'-**GCGAATTCT**CAATCAGTAGGCTTCGT-3' (ORF stop codon is indicated in bold type; underlined sequence is a *Eco*RI site). The Expand High Fidelity PCR system (Roche) was employed to minimise potential PCR errors. The amplified product was gel purified, restricted with *Eco*RI and *Bam*HI and cloned into the corresponding sites behind the galactose-inducible GAL1 promoter of pYES2 (Invitrogen) to yield the plasmid pYDESN. The fidelity of the cloned PCR product was checked by sequencing. The vector pYDESN was then transformed into *S. cerevisiae* strain Invsc1 (Invitrogen) by a lithium acetate method, and transformants were selected on minimal medium plates lacking uracil.

For the feeding experiment with PUFAs, cultures were grown at 22°C in the presence of 2% (w/v) raffinose and 1% (w/v) Tergitol NP-40 (Sigma). Expression of the transgene was induced when OD_{600nm} reached 0.2-0.3 by supplementing galactose to 2% (w/v). At that time, the appropriate fatty acids were added to a final concentration

of 50 μ M. Incubation was carried out at 22°C for three days and then 15°C for another three days. For the feeding experiment with saturated fatty acids, a single Invsc1 colony transformed with pYES2 (empty plasmid, control) or pYDESN was inoculated in 10 ml of minimal media minus uracil containing 2% raffinose and 5 grown overnight at 30°C with shaking (300 rpm). After 16-24 h, cells were collected by spinning at 4500 rpm for 10 min. After discarding the supernatant, the cell pellet was resuspended in the same medium mentioned above supplemented with 2% galactose and 1% tergitol, to obtain a cell density of 5×10^7 cells/ml. Fifteen ml of this cell suspension were added to a 100 ml-flask with or without addition of saturated 10 fatty acids (as mentioned in the text) at 500 μ M final concentration. Desaturase induction was then carried out at 20°C with shaking (300 rpm) for three days.

Fatty acid analysis

15 Microalgae or yeast cells were harvested by centrifugation. Total fatty acids were extracted and transmethylated as previously described [4]. Most FAMEs were identified by comparison of retention times to a 37 FAME mix (Supelco). PUFA FAMEs were also identified by comparison to a sample of standard Menhaden oil (Supelco) transmethylated as per the samples.

20 Dimethyl disulphide (DMDS) adducts were used to determine the double bond position in identified and unidentified monounsaturated FAMEs. These were made by adding together 50 μ l DMDS (Aldrich), 100-1000 ng FAMEs dissolved in 50 μ l hexane, and 5 μ l 50 mg ml^{-1} iodine in diethyl ether. This solution was heated at 40°C 25 for 15 h and partitioned with 200 μ l hexane and 100 μ l 5% (w/v) sodium thiosulphate. The hexane phase was removed, dried under vacuum, reconstituted in 50 μ l fresh hexane and used for GC-MS analysis. A Trace GC 2000 (ThermoQuest) fitted with a 30 m x 0.25 mm x 0.5 μ m film thickness ZB-1 column (Phenomenex) was used to chromatograph 2 μ l DMDS adducts injected at 250°C and a 50:1 split 30 ratio with He as carrier gas at 0.6 ml min^{-1} in constant flow mode. The oven program was 120°C for 1min then to 340°C at 5°C min^{-1} . Mass spectra were obtained using a

GCQ (ThermoQuest) mass spectrometer operating in full scan mode over 50-500 m/z. Picolinyl esters were also made from FAMEs to confirm their identities. These were obtained by adding 15 μ l freshly prepared 2:1 (v/v) 3-(hydroxymethyl)-pyridine (Aldrich): potassium *tert* butoxide 1 M solution in tetrahydrofuran (Aldrich) to 50 μ l 5 FAMEs dissolved in dichloromethane. This solution was heated at 40°C for 30 min and partitioned with 200 μ l hexane and 100 μ l 2.5% (w/v) sodium hydrogen carbonate. The hexane phase was removed, dried under vacuum and reconstituted in 50 μ l fresh hexane. Picolinyl esters were injected and separated by GC-MS using the same conditions as for DMDS adducts; Sperling P., Zahringer U. and Heinz E. 10 (1998) A sphingolipid desaturase from higher plants. *J. Biol. Chem.* 273, 28590-28596; Sperling P., Libisch B., Zahringer U., Napier J.A. and Heinz E. (2001) Functional identification of a D8-sphingolipid desaturase from *Borago officinalis*. *Arch. Biochem. Biophys.* 388, 293-298; Whitney H.M., Michaelson, L.V., Sayanova, O., Pickett J.A. and Napier, J.A. (2003) Functional characterization of two 15 two cytochrome b5-fusion desaturases from *Anemone leveillei*: The unexpected identification of a fatty acid Δ 6-desaturase. *Planta* 217, 983-992; each of which are incorporated by reference.

EXAMPLE 1

20 Identification and phylogenetic analysis of putative *T. pseudonana* desaturase sequences with other functionally characterised desaturases

Tblastn searches with 13 functionally characterised desaturases revealed 427 non-redundant raw sequences with E values less than 0.001. Twelve unique contigs were assembled after retrieving these sequences and gene models were constructed 25 manually based on sequence homology. These 12 gene contigs were arbitrarily designated *TpdesA* to *TpdesL*. All 12 showed significant sequence similarity to query sequences with 9 containing near full length open reading frames compared to other known desaturases (Fig. 1A). Interestingly, the predicted amino acid sequence of all nine *T. pseudonana* desaturases have a characteristic fused cytochrome b5 haem-binding domain (HP[G/A]G) at their N-terminus and three histidine boxes (H[X]3-30 4H, H[X]2-3HH AND Q[X]2-3HH) with the replacement of the first histidine by

glutamine in the third histidine box in all but two of the predicted proteins (TpDESA and TpDESB). These are common characteristics of a large subgroup of front-end acyl group desaturases [21]. These histidine-box motifs are critical for desaturase activity, most likely because they serve to coordinate the diiron-oxo component of the active site. Three remaining sequences (TpDESD, TpDESL and TpDESH) appear to be partial, covering only the C-terminal end of desaturases, but nevertheless they do contain a typical third histidine box of the above mentioned subgroup of desaturases (Fig. 1A).

10 In order to gain insight into the relationships of these *T. pseudonana* sequences to other functionally characterised desaturases and especially algal desaturases, we constructed an unrooted phylogenetic tree using a Fitch-Margoliash method with statistical confidence measured by bootstrap analysis (Fig. 1B). Relationships of four putative *T. pseudonana* desaturases are in well supported (>70% bootstrap value) 15 subgroups with at least one functionally characterised desaturase from other species. Both TpDESM and TpDESO grouped with PtDEL5, a $\Delta 5$ -desaturase from another diatom, *P. tricornutum* [5], suggesting these two enzymes may also have a $\Delta 5$ -desaturase activity. Similarly TpDESK is grouped with two $\Delta 4$ -desaturases TFAD4 and EgDEL4 from *Thraustochytrium sp.* ATCC21685 [22] and *E. gracilis* respectively. TpDESI grouped with PtDEL6, a $\Delta 6$ -desaturase from *P. tricornutum*. This indicates that TpDESK and TpDESI may have $\Delta 4$ and $\Delta 6$ -desaturase activities respectively. However, as enzymes with different regioselectivities are also found in a well supported subgroup (EgDEL8, CeDEL5 and CeDEL6; $\Delta 8$, $\Delta 5$ and $\Delta 6$ -desaturase respectively) and regioselectivity may even derive independently after a 20 more recent duplication (CeDEL5 and CeDEL6) [23] predictions based on homology can be misleading and it is essential to functionally characterise each enzyme.

25

The remaining five *T. pseudonana* sequences fall into three separate subgroups (TpDESE; TpDESA and TpDESB; TpDESG and TpDESN) which do not group with 30 any other known functional desaturases with high confidence. It is therefore possible

that these proteins exhibit novel regioselectivity. The current study focussed on the characterisation of one of these proteins, TpDESN.

EXAMPLE 2

5

Temporal expression of TpDESN gene

RT-PCR analysis of *TpdesN* transcript was conducted at different stages of algal growth in order to establish if and when this gene is expressed. After RNA extraction 10 and cDNA synthesis, *TpdesN* specific PCR products were amplified. PCR amplification of the 18S rDNA gene was performed as a control for the quantity of cDNA used during PCR reactions. Figure 2 shows that the diagnostic 519 bp cDNA amplification product expected for *TpdesN* was present at similar level at the different stages of cultivation of the microalga cells. Thus, *TpdesN* is 15 transcriptionally active at a constitutive level during *Thalassiosira* growth, suggesting that it may encode a desaturase with a housekeeping function.

EXAMPLE 3

Functional characterisation of TpDESN in yeast

The putative desaturase sequence annotated *TpdesN* was contained on a genomic DNA contig of 2580 bp on which no introns was detected. To establish the function of the protein encoded by this gene, the full-length sequence was amplified from 25 genomic DNA. An alanine codon containing a G as the first letter was added immediately downstream of the start codon of *TpdesN* to ensure optimal translation in yeast [24]. The *TpdesN* ORF is 1434 bp long, and encodes a 477 amino acid protein TpDESN (Fig. 1C), having a molecular weight of 53.8 kDa. Analysis of the secondary structure of TpDESN using SOSUI software (http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0.html) [25] predicted four transmembrane regions (not shown). Alignment of TpDESN with functionally characterised desaturase sequences mentioned above indicated an overall identity of 30

25%, with the cytochrome *b5*-like domain and the three conserved histidine-rich motif areas showing greatest homology.

The primary sequence of TpDESN exhibited typical features of front-end desaturases involved in PUFA synthesis. In order to characterise the specificity of this protein, PUFAs (18:2 Δ 9,12; 20:2 Δ 11,14; 20:3 Δ 8,11,14; 22:4 Δ 5,8,11,14; 18:3 Δ 9,12,15; 20:3 Δ 11,14,17; 20:4 Δ 8,11,14,17; 22:5 Δ 7,10,13,16,19) were first fed to the host yeast transformed with pYDESN and the vector alone (pYES2) as a control. Unexpectedly, after six days of incubation, TpDESN did not desaturate any of the supplemented PUFA substrate. Furthermore, there did not appear to be any production of 18:2 Δ 9,12 from endogenous 18:1 Δ 9. However, a significant increase was observed for a peak eluting in the range of sixteen carbon monounsaturated FAMEs in the yeast transformed with pYDESN (Fig. 3). The position of the double bond in this product was determined by GC-MS analysis of FAMEs derived to 15 DMDS adducts [26] and picolinyl esters. The DMDS adduct of 16:1 Δ 9 FAME yielded two major fragments at *m/z* 145 and 217 (Fig. 4A). Fragmentation of the increased FAME peak found in unfed or fed yeast transformed with pYDESN produced two diagnostic fragments at *m/z* 117 and 245 (Fig. 4B). This fragmentation pattern was indicative of an Δ 11 monounsaturated sixteen carbon FAME, 16:1 Δ 11, suggesting that TpDESN encoded a new Δ 11-desaturase. Small amounts of this FA have also been measured in *Thalassiosira* cells (Table 1). To further substantiate these results, yeast transformed with pYDESN and the control empty vector, pYES2, were cultivated in medium supplemented with saturated FA (14:0; 16:0; 18:0) representing potential substrates for the synthesis of the monounsaturated product. 20 Yeast fatty acid profiles were analysed after three days of incubation at 20°C. Results in Table 2 showed that a small amount of 16:1 Δ 11 (0.23% of total FAs) was detected in yeast transformed with pYES2, suggesting endogenous synthesis of this FA from 25 16:0. This FA accumulated at a higher level in both types of transformed yeast after feeding with 14:0, with values up to 5.84% in pYDESN transformants. A possible explanation for this increase in the pYES2 transformants is that the endogenous yeast Δ 9-desaturase was able to use additional 14:0 to produce 14:1 Δ 9 that was 30

subsequently elongated to 16:1 Δ 11. Moreover, it has been reported that wild type yeast cells cultivated in media supplemented with 14:1 Δ 9 synthesised 16:1 Δ 11 by Elo1p-dependent carboxy terminal elongation [27]. After 18:0 supplementation, the percentage of 16:1 Δ 11, of about 6% total FAs, was similar to that observed after 5 feeding with 16:0. Presence of extra 18:0 could lead to an inhibition of the 16:0 chain elongation system, which might allow more 16:0 to be available for Δ 11-desaturation. On the other hand, 18:1 Δ 11 represents 1.2% of the total FAs in transgenic yeast. No variation in its proportion was monitored under the different 10 conditions of incubation, even after supplementation with 18:0 in pYDESN transformants. This suggests that this FA originates from elongation of 16:1 Δ 9 rather than Δ 11-desaturation of 18:0.

EXAMPLE 4

15 Functional characterisation of TpDESI in yeast

To establish the function of TpDESI, the full-length cDNA was expressed in the yeast Invscl under the control of an inducible galactose promoter. Potential substrates of front-end desaturases (18:2 Δ 9,12; 18:3 Δ 9,12,15; 20:3 Δ 8,11,14; 20:4 Δ 8,11,14,17; 22:4 Δ 7,10,13,16; 22:5 Δ 7,10,13,16,19) were tested. Figure 12A and 20 12B show that after supplementation of the medium with 18:2 Δ 9,12 and 18:3 Δ 9,12,15 respectively, and after three days of incubation, yeast cells containing pYDESI had extra fatty acids. Extra peaks observed when cells were fed with 18:2 Δ 9,12 had a retention time identical to 16:2 Δ 6,9, 18:2 Δ 6,12 and 18:3 Δ 6,9,12 (Figure 12A). Extra 25 peaks observed when cells were fed with 18:3 Δ 9,12,15 had a retention time identical to 16:2 Δ 6,9, 18:2 Δ 6,12 and 18:4 Δ 6,9,12,15 (Figure 12B). These results demonstrate that *TpdesI* encodes a Δ 6-desaturase which can introduce double bond in exogenously fed 18:2 Δ 9,12 and 18:3 Δ 9,12,15 fatty acids, but also in endogenous 16:1 Δ 9 and 18:1 Δ 9 fatty acids. Percentages of conversion of these different 30 substrates are given in Table 3.

Fatty acid profiling of marine microalgae had shown that *T. pseudonana* represents a good candidate to discover genes involved in the production and storage of PUFAs [4]. Analysis of the recently completed draft genome of this microalga revealed the presence of many candidate genes for elongase and desaturase activities most probably involved in catalysing different steps of the PUFA biosynthetic process. We have identified 12 possible desaturase genes, 9 of which there is sufficient sequence information to demonstrate that they exhibit typical features of front-end desaturases, i.e. a cytochrome *b5* domain in the N-terminus and three histidine clusters located at highly conserved regions. Phylogenetic analysis revealed that several of the genes are closely related to a number of previously characterised front-end desaturases involved in PUFA synthesis. However, the current work highlights the fact that desaturase function, in terms of regioselectivity, cannot solely be based on prediction from primary amino acid sequence homology.

15 The fatty acid profile of *T. pseudonana* cells is quite diverse (Table 1), with the health beneficial EPA (20:5Δ5,8,11,14,17) and DHA (22:6Δ4,7,10,13,16,19) accounting for a large proportion. However, the number of desaturase gene sequences found in the genome was higher than we expected based on the number of different desaturation reactions required to produce the diversity of FA in this microalga. This 20 suggested that non-obvious desaturation reactions might also occur in the *Thalassiosira* cells. As a first step to establishing function of the many putative desaturase sequences, we focused on the *TpdesN* contig due to the fact that the sequence was full-length and intronless. A temporal expression study showed that *TpdesN* was constitutively transcribed during algal cultivation. Expression of the 25 *TpdesN* ORF in yeast supplemented with PUFAs as potential substrates for desaturation revealed no new products. There was also no evidence of activity with the endogenous 18:1Δ9 which excludes the possibility that TpDESN acts as a Δ12-desaturase. However, an increase in the peak area of a FAME eluting in the range of the sixteen carbon FAMEs was identified and GC-MS based analysis revealed this to 30 be 16:1Δ11 fatty acid. Small amounts of this FA are also present in wild type yeast. However, quantitative comparison of FA levels in the empty vector pYES2 and

pYDESN transformants showed that proportions of 16:1Δ11 increased in the presence of *TpdesN* in both unfed cells and cells that had been fed different saturated FA. No other changes in either peak area or new peaks were detected in pYDESN transformants, indicating that TpDESN is specifically involved in conversion of 16:0 5 to 16:1Δ11.

The presence of small amounts of 16:1Δ11 have previously been reported in many microalgae, including *T. pseudonana*. However, a function for this FA in algal cells has not been established. The low quantity observed in many marine microalgae 10 suggests that it may act as an intermediate in an as yet unidentified biosynthetic pathway. In insect cells, 16:1Δ11 represents an important precursor for pheromone synthesis, where it is produced by an acyl-CoA Δ11-desaturase. Interestingly, the insect Δ11-desaturases do not possess a cytochrome *b5* domain in their N-terminal region. This represents a major primary structure difference compared with TpDESN. 15 The cytochrome *b5* domain is not a determinant of the substrate specificity [28]. Alignment of the desaturase domain of TpDESN with the full sequence of insect Δ11-desaturases showed an identity of 20% (data not shown). In insect cells, Δ11-desaturases are more or less specific depending on the origin of the sequence and well-documented reviews exist on this subject [14,15].

20

In conclusion therefore, although the TpDESN primary sequence is very similar to front-end desaturases, it should not be considered a member of this family of desaturases because it acts only on 16:0. Identification of such a novel enzyme expands the functional repertoire of the membrane-bound desaturases and it should 25 provide useful comparative information for understanding phylogenetic relationships between these enzymes. One question that remains to be answered regards whether cytochrome *b5* was independently fused to desaturases that had already acquired their different specificities, or whether an ancestral fusion protein for proximal lipid modification duplicated and subsequently evolved into different desaturases. Studies 30 of the primary structure of the different PUFA desaturases support the fact that enzyme conversion (i.e. change of specificity) can be achieved through a relatively

few structural changes [29]. The high degree of homology between the many potential front-end desaturases identified in the genome of *T. pseudonana* support this notion. Given the FA profile of *T. pseudonana* cells and the complexity of the desaturase gene family it is likely that different genes will encode $\Delta 4$, $\Delta 5$ and $\Delta 6$ desaturases. It will now be very interesting to functionally characterise these remaining putative desaturase genes and study the relationship between regioselectivity, primary amino acid sequence and phylogenetic relationship. A crystal structure for these enzymes is still not available due to technical difficulties in obtaining sufficient quantities of purified membrane-bound protein. Molecular 5 genetic approaches involving site-directed mutagenesis have provided new insight into structure-function relationships, including for example that residues in close proximity to the histidine motifs have been found to be involved in shifting the ratio of desaturation/hydroxylation activities [30]. Detailed comparative analyses and computer modeling of these diverse desaturases from *T. pseudonana* may further 10 guide site-directed mutagenesis studies aimed at defining key residues controlling substrate specificity and regioselectivity of the introduced double bond.

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Claims

1. A transgenic cell comprising a nucleic acid molecule which comprises a nucleic acid sequence which nucleic acid molecule consists of the sequences represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b, or nucleic acid molecules which hybridise to these sequences, wherein said nucleic acid molecules encode a polypeptide which has desaturase activity.
5
2. A cell according to Claim 1 wherein said hybridisation conditions are stringent hybridisation conditions.
10
3. A cell according to Claim 1 or 2 wherein said nucleic acid molecule comprises the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b.
15
4. A cell according to Claim 3 wherein said nucleic acid molecule consists of the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b.
20
5. A cell according to any of Claims 1-4 wherein said cell over-expresses said desaturase encoded by said nucleic acid molecule.
6. A cell according to any of Claims 1-5 wherein said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 10a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 11$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 10a and encodes a polypeptide with $\Delta 11$ -desaturase activity.
25

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7. A cell according to any of Claims 1-5 wherein said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 8a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 6$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 8a and encodes a polypeptide with $\Delta 6$ -desaturase activity.

10 8. A cell according to any of Claims 1-7 wherein said transgenic cell is a eukaryotic cell.

9. A cell according to any of Claims 1-7 wherein said cell is a prokaryotic cell.

10. A cell according to Claim 8 wherein said eukaryotic cell is a plant cell.

15 11. A plant comprising a cell according to Claim 8.

12. A seed comprising a cell according to Claim 9.

13. A plant or seed according to Claim 11 or 12 wherein said plant or seed is an oil seed plant.

20 14. A reaction vessel comprising at least one polypeptide encoded by a nucleic acid molecule wherein said nucleic acid molecule comprises a nucleic acid sequence consisting of the sequences as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b, or nucleic acid molecules which hybridise to these sequences, and which encode a polypeptide which has desaturase activity; fatty acid substrates and co-factors wherein said vessel is adapted for the desaturation of said fatty acids substrates.

25 30 15. A vessel according to Claim 14 wherein said polypeptide is expressed by a cell according to the invention.

16. A vessel according to Claim 15 wherein said cell is a yeast cell.

17. A vessel according to Claim 15 wherein said cell is a prokaryotic cell.

5

18. A method to desaturate a fatty acid substrate comprising the steps of:

- i) providing a reaction vessel according to any of Claims 14-17; and
- ii) growing said cells contained in said reaction vessel under conditions which allow the desaturation of at least one fatty acid substrate.

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ABSTRACT

We describe transgenic cells transformed with nucleic acid molecules which encode enzymes with desaturase activity and the use of these cells and enzymes in
5 biocatalysis.

10



Table 1
Composition of the major fatty acids in *T. pseudonana*

Fatty acid	Mol % of total fatty acids
14:0	11.50
16:0	17.95
16:1Δ9	19.81
16:1Δ11	0.19
16:2Δ9,12	2.47
16:3Δ6,9,12	6.68
18:0	0.47
18:1Δ7	0.26
18:1Δ9	1.50
18:1Δ11	1.52
18:2Δ9,12	2.37
18:3Δ6,9,12	0.98
18:3Δ9,12,15	0.32
18:4Δ6,9,12,15	5.72
20:0	0.44
20:3Δ8,11,14	0.26
20:4Δ5,8,11,14	2.46
20:5Δ5,8,11,14,17	17.51
22:6Δ4,7,10,13,16,19	6.64
24:0	0.49

Microalgal cells were harvested during the exponential phase of growth as described in section 2. Fatty acid were extracted and converted to their methyl esters before analysis by GC. Each peak was identified by the retention time compared to known standards. Each value represents the average of triplicate measurements within a representative culture.



Table 2

Composition of the major fatty acids of pYES2 and pYDESN yeast transformants with and without addition of exogenous saturated fatty acids

Fatty acid	Mol % of total fatty acids							
	- substrate		+ 14:0		+ 16:0		+ 18:0	
	pYES2	pYDESN	pYES2	pYDESN	pYES2	pYDESN	pYES2	pYDESN
14:0	0.78	0.52	1.12	0.96	0.76	0.58	0.76	0.70
14:1Δ9	0.22	0.13	1.20	1.29	0.23	0.14	0.23	0.20
16:0	18.40	15.04	18.37	14.62	23.85	22.09	17.22	15.09
16:1Δ9	39.73	35.55	43.39	36.24	42.24	37.03	36.24	31.67
16:1Δ11	0.23	3.27	2.36	5.84	0.22	5.57	0.25	5.84
18:0	7.37	7.34	6.61	7.23	6.36	6.60	16.72	17.47
18:1Δ9	30.19	34.32	24.44	30.24	23.89	25.19	26.07	26.58
18:1Δ11	1.20	1.21	1.35	1.30	1.19	1.12	1.08	0.96
26:0	1.89	2.63	1.16	2.29	1.26	1.70	1.43	1.50

The values given are expressed as mol % of total fatty acid methyl esters quantified by GC. All values are the means of duplicates from a typical experiment.



substrate	product	% conversion	
		<i>Thalassiosira pseudonana</i>	<i>Phaeodactylum tricornutum</i>
16:1Δ9	16:2Δ6,9	14	6
18:1Δ9	18:2Δ6,9	18	5
18:2Δ9,12	18:3Δ6,9,12	54	28
18:3Δ9,12,15	18:4Δ6,9,12,15	68	27

16:1Δ9 = 16:1n-7 16:2Δ6,9 = 16:2n-7 18:1Δ9 = 18:1n-9 18:2Δ6,9 = 18:2n-9
 18:2Δ9,12 = 18:2n-6 18:3Δ6,9,12 = 18:3n-6 18:3Δ9,12,15 = 18:3n-3 18:4Δ6,9,12,15 = 18:4n-3

Both algae are Diatoms. TpDESI and Δ6-desaturase sequences are 70% identical at the amino acid level. Data for the *Phaeodactylum* enzyme have been taken from the following paper (Table 1):
 Domergue, F., Lerchl, J., Zashringen, U. and Heinz, E.
 Cloning and functional characterization of *Phaeodactylum tricornutum* front-end desaturases involved in eicosapentaenoic acid biosynthesis.
 Eur. J. Biochem. 269 (16), 4105-4113 (2002)



Figure 1

Introducing the 3S haem-binding domain

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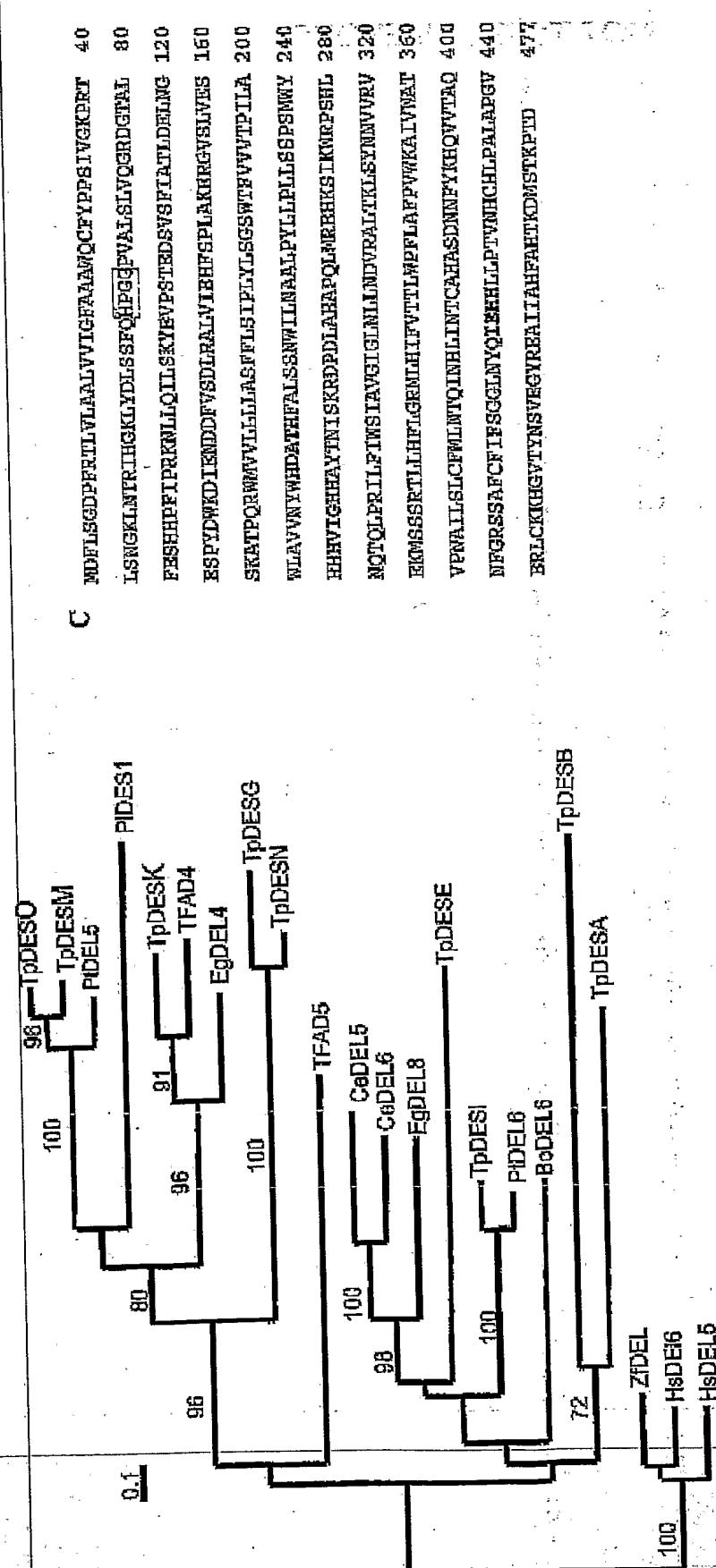
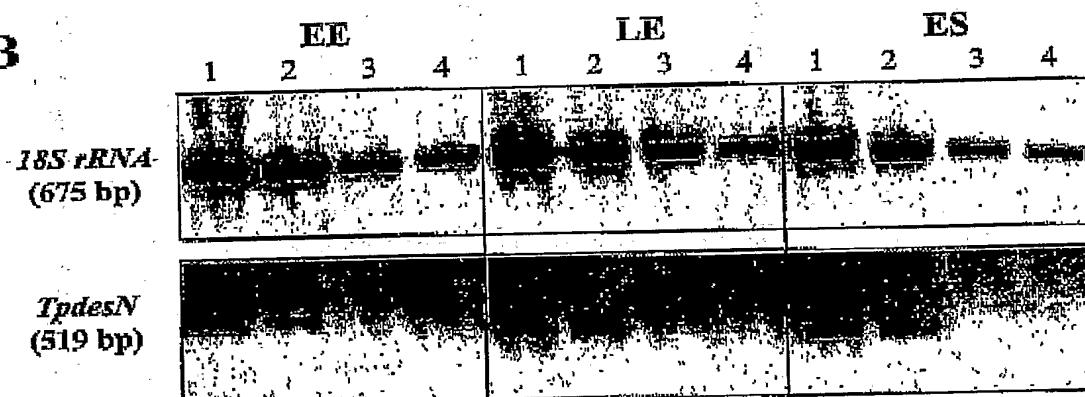


Figure 2

A

Growth phase	early exponential: EE	late exponential: LE	early stationary: ES
Incubation time	142 h	237 h	311 h
Nitrate degraded	20%	60%	100%

B

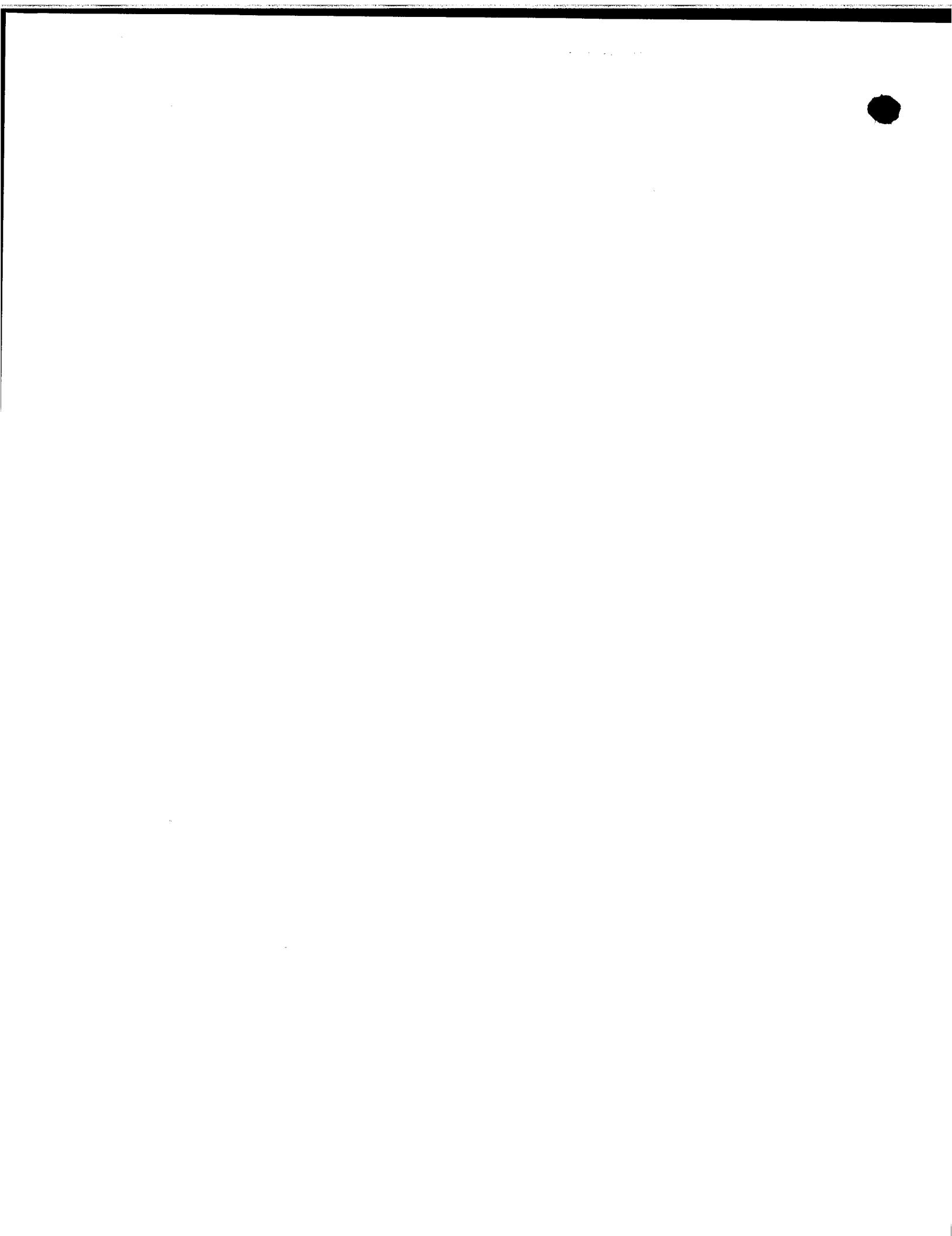
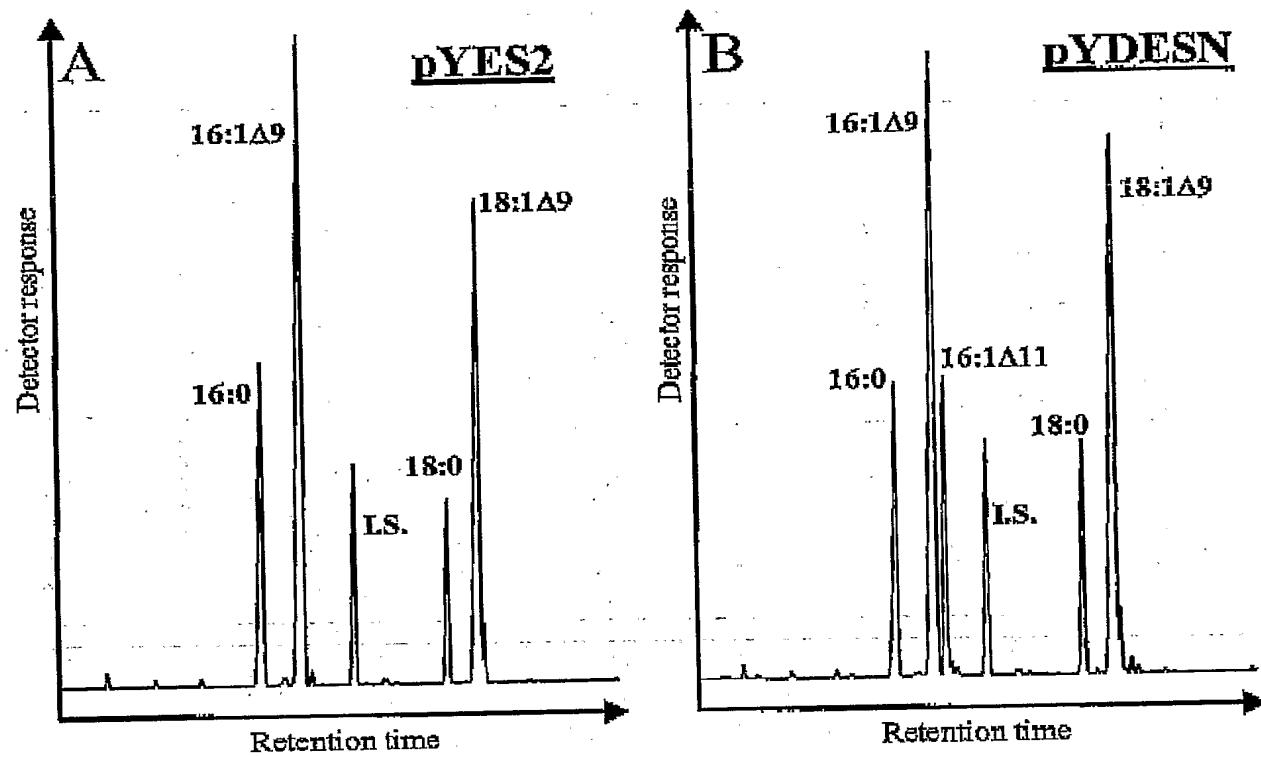


Figure 3



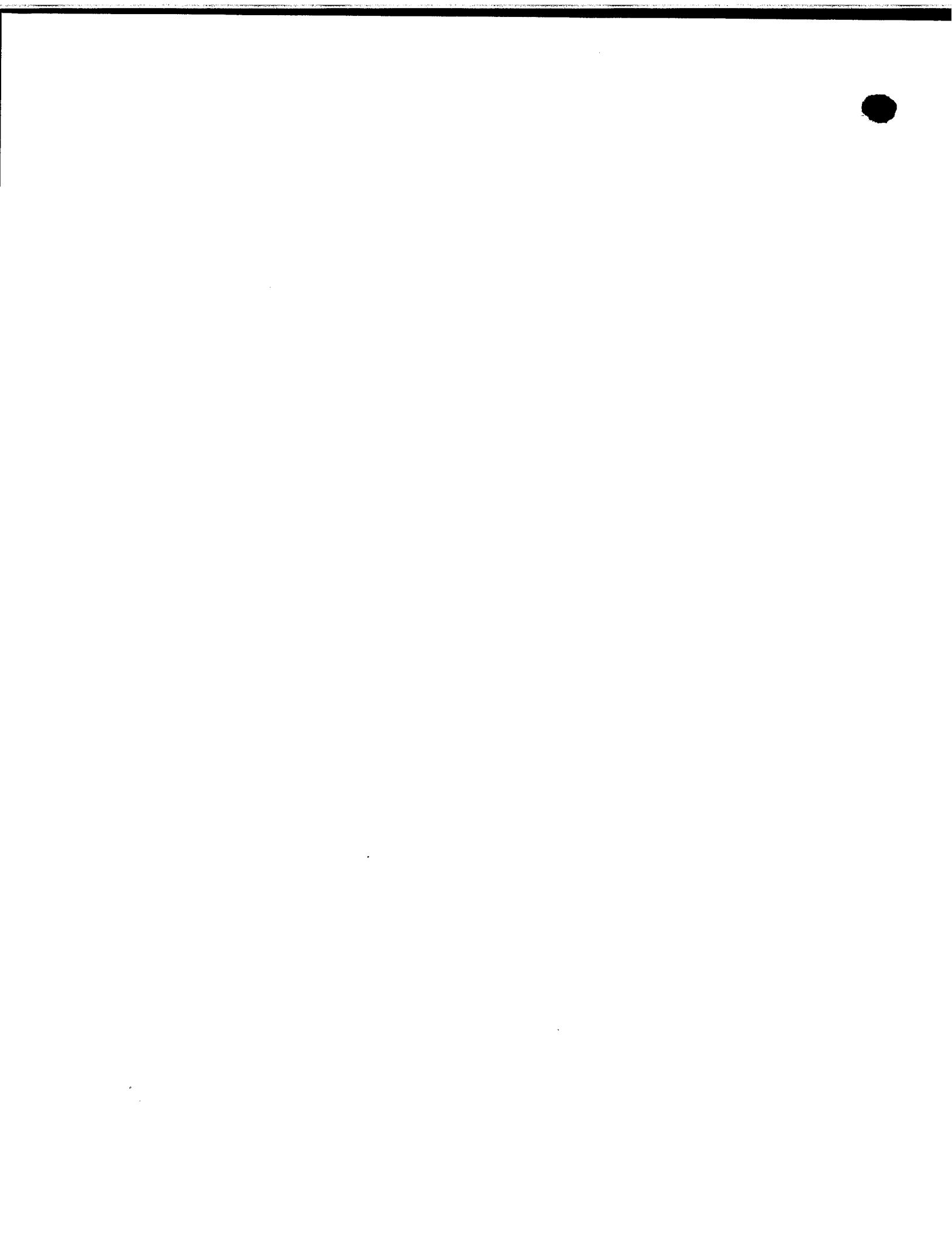


Figure 4

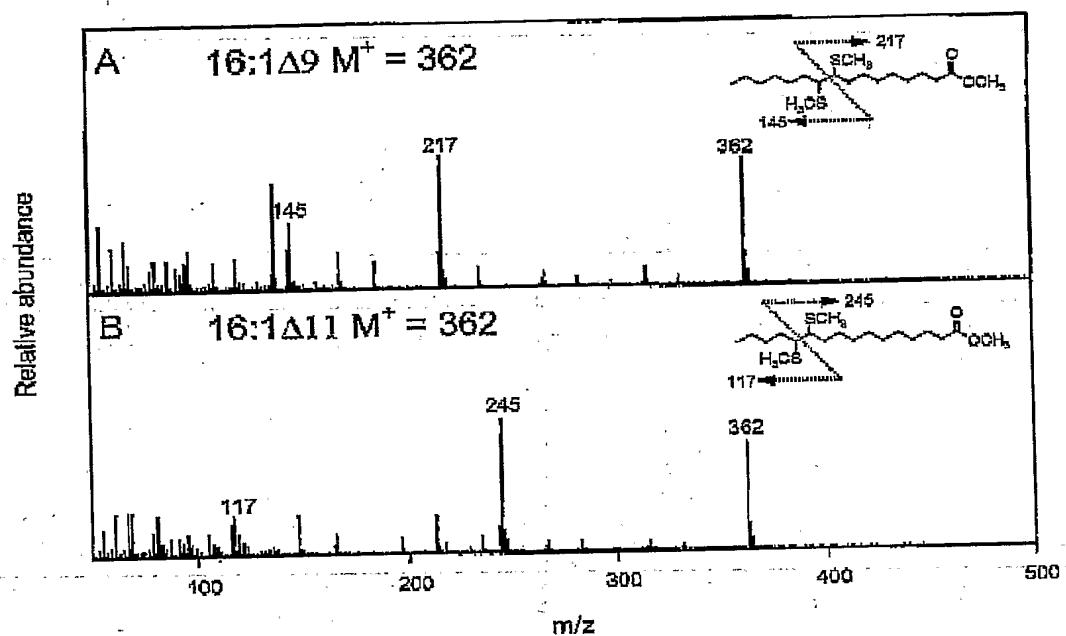




FIGURE 5atpDESA = Thalassiosira pseudonana DESATURASE A

CGGAGGGCGCAGTGGAGAGGTTCCGGAGGTTTGATGTAATTGGAGGTGGGTCAAATAC
 AGATTCTGCCCTAACATTTCGGAAATTGGCTCAGITTGATTCAGGAGGAGGCGCT
 CGGCAGGAGGGCCCGTACACCTTTGCAATTGGACTTCAGTGGTTCTACATTTTT
 CCTTCTGGAACCCAAACGCTGTCCTCAATTCTCCCTCCATACTCACCGATGGATCCC
 CGAAAATGCCACCACCAATCACCCCTGTCATCNCAAACCTCGTCATCCTCACATTTTC
 TTAGGACCAATTGGCGGTGACCCCTCCCCGCACTGCCAGTCTATGGGTCACTATCT
 CCCACATTGGAGAGGTTATGCTAAACGTGTCATCATACATATGATAACTGGAGAGTG
 CACACGAAGAGATCAATGCTTGAGCTAGGAGGGCGTATGGCTGTGAGCCGGAGCTTT
 CACTTAAGATATTACGGCACGGCAAGTCTACTCGACAATACAACCGATGTCAGGTTTA
 TGCAATAGCTCAAGTGTATCAACACAAACGTGCCAGAATGACGACAGTGCCTACGAA
 GATGATGCCATGAGAGCTTTGGCATTGCTATGGCGTGTGAGCTGGGAAACGTTGGTC
 GGTGCGTTAGTGTAGGAGTGCATTCAGTGGAGATAACCTGACAAACAAATCT
 GCGATACCAACAAATCTCAGGCAAGCCAAATATCTGATCAAAAGCAGCTGTGACA
 TCCGGCAGTACCTGCGCTGAGAGAGAAGGCACCAAAAGACGGTCAAGTACTCCTCGAT
 CGCAACTGGTACAACGTTGAAAAGTTCGTCCATGATCATCTGGAGGTGTAGAAAGTGTG
 GAGCAGTATCTGGGGCAGATACTCTGTTGTTAGAGTGTGATGCAAGAAACTTCAACT
 CAATCATGAAATATGCAAGCCGGTACGGAGCTGCCACCCAGAGAAGAAACTTGAGGCTCTC
 ACAAGCCCGCTCAAGAGGTTTGTCTGATATGATGGAGACTTTGTTACCAATTCCATT
 GATATCGCTTCTCCAGAAATGCTTCCAAGCCACGGCACTTGTGACCTGAAACTCATTTGAG
 AAGGACTTCATGACTTATATGAAAGAGTTGCTCAGGGATACTTCAGGCCCTCAACA
 ACATGGCTACTCTGGAACACAGGGTACTGATAGTATCATCGCTATCTGTCATCTCA
 ATGAAAGTGTCTACCACCAACTTCGTTGCTTAACCTGGAGCATTGCTTGGCTCTTTG
 CACCAAAGTGGATTCTCCTAATGACGATGCCAGGACACCCATAATTGGCTGGAAACGAACGG
 CTGAATGACATTGGTGGATCTATGGCACTGCTTCTGGCTGTCAATGGCGCTTGG
 TGGAGAGAGGAGCATAGAGAACATCATGCTTCTCAACACTTACGGATGAAAGTGGT
 TTCAAAAGTCTCCCAGGTGTGTCAGCGTCACTGTAGACGGACTTCAAAGTTACTTGTCTC
 TCGTGTCTACACATTGCAATTGCAATTGCACTCACAGATGAGAGAGGAGCTCTGGATA
 CAGAACAGAAAGTTGATTCGGTCTTCGGTGACGAGATCAATTCTTAACAAACTTT
 CAGCACATTGTTCTCCGATCATCTTATGGTGGCCGGTGTGTTAGTGTCTAG
 TCTACACTGACTGACAGGAAGTTGGTCTGGAGTAAGTGTCAATTGGTATTGAG
 AAGGAAGTGTGAAATTGACTTCAACTAACTGCACTGCCACCTCATCACGACGAT
 AGCAAAACTTGCTAATGTTGTCAATATCCTACTACACTACGCAATCTTATCTCAGACGAG
 TCGTCTATCCCCGTGTCATCATCGGCTCTCTGGCAAGCTATTCTCTCTTGTCAATT
 GCTTGGAAATCACTACGTCAAGCCTGGAAATGACTCAACGATGCCACAGAGGAAACTT
 CTGCGTTGGCAGATACTAACGACTCAAGACTTTGCACTGTCACGTTGGCTCTGGCT
 GTACGGAGGTCTCAACTTCACTATTCCCATCTATTCCCAACGTTGCTAGAGACTA
 CTTTCACATTACATCACCCACGGCATTGGGTGAGTGTGCTGTTAGTGTGCTACATTCA
 TATCAATGATAACTCATGACTCCATTCTTCCACAGAGACTATGTGAGAACGGCCTT
 CCGTTATTGAGATTGGCTCATGGTGTGCTGTTGGAATGGTCACAAACTTAAAGAA
 GTGAGGAAAGACTTCGCAAGGAAAGCCACGGAGTGTGGCTTCAATGTAACCTGATCT
 TAAGTGTGCAAGGATACTGAGGTTGATATTACTGTTTGTCACTGAGTACTTGTCTAA
 TATGATGTTAGCAACCGCAGCTGGAAATTAGTTAGTGTACTATGTAACGAAAGTT
 ACGTGCACTCTCTGCACTCATCATCGTGTGAAAGCCATTGGCTCAAGAAGTATCC
 TAATCCCCGCAACCAAAACGACTCTCGTCTTACCCCATCTTATCAGCCGCTCTACCCG
 ATGTTGGCTGTTGCTGGGCTGCTGCTGAACCCCTGGCCGGGATAATGGCAAGGGCA
 GTGGACACACTGATAATCTCTTCACAGAGTTATGAGCTGGGTGTTGTACCAAAACCT
 CCTTATATGGTACTTAATGGACCCGGTGTCAATTGCTGGCCGGTGTGACCGTTG
 GACCGATAGGTGGCCAAAGGCCACACAGAAGGCACTAAAGCCGAGCCTTGAGGAA
 ACTCAAGAAACCCGATGCTCCACGTTAAAC



Figure 5b

Full length cDNA sequence: GCT (Alanine) codon add by us to improve yeast expression

ATGGCTAGAGCTGTTGGGCAATGCTATGGGCGTTGCGAGCTGGGAAACGTTGGTGGCTGGTTGT
 GCGTTAGTGTAGGAGTCGATCTTCAAGTGCAGATAACCTACCCAAACAAATCTGGCAATA
 CCACARAAATCTTCARAAAGCAGAACAAATATCTGATCAAAAGCAGCTGGACATTCGGC
 AGTACCTGCGCTGAG
 TGGTACCTGAGCTGAAAGTTCGTCATCATCATCTGGAGGTGAGAGAGTGGAGAG
 TACCTGGGGCAGATACTCGTTGTTAGAGTGTGATAGAAATCCAAACTCAAATC
 ATGAAATATCGCAAGCCGGTACGAGCTGCCACCCAGAGAGACTTGAGGCTCTCACAGH
 CGCCGTCAGGAGGTTGCTCTGATATGATGGAGCAGTTGAGGAAATTGATATACTC
 GCTTCTGAGAAATGTTCCCAACGGCAGTTGACCTGAGTGTGAGAAAGAAC
 TTCACTGACTTATGAGAGAGTTGTTCTGAGGATACTTCRAGCCCTCACACATGG
 CTACTCTGAAACACAGGGTACTGATTGATCATCGGGTAACTGTCATTCAGAA
 GTGCTTACCRACCAACTTGTGTTGCTTACCTGGACCATCTGGCTCTTGGACCAA
 ATGTTGAACTCTCATGACGATGGCAGGACGGATAATTGCTGCTGAAACGAAACGGCTGAAT
 GACATTGTTGGATATGGCACTGTTCTTGGGTGTCAATGGCCTGGGGAGA
 GAGGAGCATAGAGAACATCATGCTTCTCARACATTAACGATGAAAGTGGTTCAAA
 GATCCCCGAAATGAGAGAGGAGCTGGATACAGAACTGAGAAATTGATTCCTTGGT
 GACGAGATCATTCATTCATTCATTCATTCATTCATTCATTCATTCATTCATTC
 ATCGTTGGCCGTTGGTAAATTGTCGTAATTTTACACTTGAGAGGAATTCCGGTCT
 TGGACAAACTTGGTAATGTTGTCAATTCATTCATTCATTCATTCATTCAGACG
 AGTCGTTCTATCCCGTCTACATCATGCTTCTCTGGCAAGCTATTCTTGTGCAA
 TTGCTTGGGAACTACTGCAAGGCTTGGAACTAGACTCAAGCTTGGCTTGGCTGG
 TTCTGGCTTGGCAGACTTARGCAACTTTCATATTCCCATCATCTGTTCCCACTGTTGAGAG
 TACTTTCACATTACATCACCAAGCAATTGGAGACTTGTGAGAAAGCAGGGCTTGGCTT
 ATGAGGATTGGCTTATTGATTCGTTTGTGAACTGTCAACACTTAAACAGTGGAGG
 AAAGACTTCCGCTACGAAAGCCACGGGAGTGTGGCTTTCATCATACGTTA

Figure 5c

Amino acid sequence deduced from cDNA sequence

MARAWALLW ALQLSTLVGC ALVLGVHIF S CDNLKOSAI PTKSHAKAPI S DQKAAVTSG
 STCAVREKAR KDCLVLLDGN WYNVEKFVH HPGGVEULEQ KLGADISFVF RYVHRNPTQI
 MKYTRIPVRAA TPEELALTS PRQEVLDDM DDFVTEWSDI ASPEMLEKPT QFDLMSPBD
 FIDLYEEFVVA QGVFKFSTTW LLIWNTAVLIS IIAALSVISNK VLPPTISFVLS GAILGLFWHQ
 SGFLMHDAEH BNLAGNERLN DILGWTIYGTIV FLGUNGAWWR EEEHREHHAFL NTYDDESGFK
 DPQMRLEDVNI QMKLLEPPFG DEIIEHFLNFQ QHLFLPIIF IVGRVGIYVD STLTERKFRP
 NTILGNVCHI LLYHAILSQT SRIPVUTIG SLWQATLSLQ LLGNNHIVVKPW NRIMIDATEGN
 FGVWQZLSTQ DZACPRWSRW LYGGLNPHYS HHLFPTLSRE VFKHTSPRIR RLCBKHGLPF
 IEIAFIDCVV GMVNNFNEVR KDPATKHSQ VAFMYT



CONTINUATION OF PAGE

Figure 6a

TpDES = Thalassiosira pseudonana DESATURASE B

NANCCATATGCCGGATAACGGCCAGGGTATAACCCACAGGCCCTCCGTTGC
 AGCAAACCTCTATCCAATACCTCCCCATGAACCCCCCTTCCGGCACCCCT
 ATATGCGAGACTCGTCTGGACCTGCAGATGATGACTGGTGAGGCCA
 AATTAGTTGGGAAATGCGTGAGATGGAGGCCTTATTCTTTGCAATCAGG
 GCGCTCGTCAAGAGGAGATCCATGTTGTTGTTGATAGGTAGAAAGAGAT
 CGAGGCGGTGATTCAACTATTCAAGGATACTGAAAGAGTTGATATAGCAGC
 AGTAATATATCTAGTTGTTGTTGTTGTTGTTGATCAAGTATTCA
 AATGACGCAACAATAACGTTGGTAGTGTATGGGTGAACAGGTGTTGGGA
 CAAAGGTTTCTATAAATCTATTAACTGTTGTTGTTAAACGACGAAAAA
 GAAGCCACTCTGACCCATTCCAGCGCAGACAAGACCAGCAGGCACAGAAC
 ACCACGGACACACCGACCCGAGCCGAAAAAGCCAACAACAGGACACCGAC
 CCGAGCCGATAAGCCGACAGGCAAAGGCTCTGCTACAAATCTACAAAAA
 CGGCAACATCAAATCATGCCACCCATCAAGACACACTCGACGGAGCC
 CTTCGTCTGCCCGCATCCACCAAGTCGCCCCACCAACCCCTCTCC
 CCCGGCGAAACCCCTCAAAACGATACTCCCCCTCCCAATCTCCCAACAC
 AACACTCCCACCGATGCAATGGCTCATTTACAAATCCAAAGTCCTTGACAT
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 TTGCGGTATGGATGCTACCGATGAAATTGAGGGCATTTCATGATGATIG
 GTTTGGAGGAAGAAGTTCGCTCATTTGTGATTGGGAGGGTGGGATTGGAC
 TACTACGGCGGGCGAGGAATACTGTCACGAAGGGATGGACAGGTTCCGG
 AGCTTATCAAGGAAATTTCAGAGAGTTGGGTGAACACTTCGACAGGTTGGGG
 TACCTTCACGTCACTGGTATTTCGTCAGTGGTAAAGGTGCTACCGTCTT
 CGCCCATTTGGATGTCACCGACTCCCTCTCAATACCGATTCCATCC
 CAGCACACATGCTCGCGGGTACTCTCGGTATATTCTGGCAACAATT
 GCATTGCTGGACATGACTGTTGTCACATGTCGGCGGGACTCATGCC
 TGATCATATCGATGTAACCTAAGCTGGAGGACACTGGTACCTTCTTCATG
 GGATTTCGGTACGGTGGTCAATGTCATCATGCTGTG
 CCAAATAGTGTGATTGAGCCGGACATTGCTCATTTGGGTGTTGC
 GTTGCATGAGCACATGTTACGTCGTTGTTAACAGTATCATGGGAGGG
 TGATGGAGTTGATTGGCTGGCGCTAATGTCCTTGCCATTCAACAC
 TTTGGTACTATCCCATAATGGCGTGGCGAGGTTCAATCTGTACATTCA
 ATCAGCATTGTTTGGCTGCAAGAACGATGGGATGCGAGGAAGAAGGG
 GATCCCTAGATGGATTGCTGGCTTCAATCGTGTCTCTGTTGGTT
 AGCGGTGCTGGTCAATGCACTCCGAGCTGGCGGGAGCGTATGGCATTG
 TCTTCGTCAGACATGCTGTACCTGGTTACTGCAATGTCATGCAACCTGTC
 GCCTTCTCTGGACAATCTGATCCCACAAAGAGGACCCGGTTGGGTGCT
 CTTTCGAAGCCCGGTCTGGCTTGGCACATGGCGTCCCGGGTCCA

Figure 6b

partial amino acid sequence deduced from the genomic cDNA sequence

MPPSIKDTID EPPFUSPASTK SPTTKPILLPR RKLPLKRYSPS QISQHNTPTP ANLITIKGVL
 DISKWLISHPRP GGEOTULIRPA GMDAIDELRA FHDWDWLKEE LPHFVIGBVD WITTTGGAAENT
 VTKOGQVSEL IKDPRLELNG PDRLGTVHVS PWTYVREKVAI VFAIFGCAICL LLFNTDSDPA
 HMLAAVLLGI FWQQFPAVUGH DCEHMSAINT ARDHIDVPLK GALVTFENGSI SVANWKRATHN
 VHHAVPNSVD CDFDIAHLPV FALHEHMFTS LFNKTHGRVM EFDWLARNVF VPFQHFWVYP
 IMAYARFNLY IQLSALPLASK NDGHAGKNGS SRLDILIAFTR VLLLVSGAGV MHPPELGAYR
 IRLRQTCCTW VTCACAITGRL LLDNLDPTRG PGWGALSERK FWAFATLASK R V



Figure 7aToDESE = Thalassiosira pseudonana DESATURASE E

CANCTAACGGGAAGAGGGCCTTATTTCACCACAGTGATAACCTTCGG
 CTGIGACCACGGGAGCAGCCGTGGGAGGCCGCGTCTGACCAGCCCTGTC
 TTTTTGGAGCATCCTCACCAACACATCGCATCTCGTGCACGGGGATCAG
 TGCACACTCTTGTCTCATTTGTTAGATGTACACGGAAAGAAGCACAATCCA
 GCCCAGCTTCTACAAACATCTCAGGACCCCTGCAAACACGCATCACATCAT
 GATGTTCCACCGAGTCGTCACTCGGCATCGCCCTCACAAATGGCTGTGTC
 CCAGTTCTCCTCGCCCGGTCAATTGGAACGTGCTTATGCAATCA
 TCCACCACCTCTCGTTTCTGACAATGATTGAAAGTCAGAGATTTCTGA
 CAGTGTCAACACGGAAAACACGGAGATGACATCACTTCTGAAATGCTTA
 CTGGCGGGAAATGCAATGAGGAAGCTGACTGGCGGAAAGTCCCTGCTTGT
 CATGACCGAGGAATGCCGTACTACTTGGATGTGAGGATTCAATGCCAAATG
 GTACGATCTTCAGGTGACTGCAAGTGTGTTATGCAATTGTTATAAGTTC
 TATTGCAATCGGCACACGGATATTGTTGTGATCAATGTTCAACAG
 CCATTGTTCTCTACTTCTCAGGATGGCGCAAGCTCACCTGCAAGG
 ACCCAGCTGGATCGACTGTAACGAGGTGAAATGTCACAGCGTCTTCCCGG
 ACGCATTTCACACCCAAAAGGACGTGAAATGTCACAGCGTCTTCCCGG
 TCTGCCUCGAAACGGCTGCGGTTCTTGAAGGCAATCTGCAAGCAGCTTACTC
 GCAGACGGAGCTTAACCTTAGGAAGTTGAGGGATCAATGGAAAGTCAAGG
 GGTGTCGGAGAGGACTTTGTCCATGAGGGAAAGTTGCTGGGATTTGG
 GCATCGTGGTACAGGAGCAGGATGACTGGGGAGAGTGTCTCTCTCT
 TTCAACTTTCTGTTGGGATTGTCTATGAGGAATGCTGGATGGTGGGGC
 ATGATTATATTCAATGGTGTGATAAGTTCAAGTTAATGAGGCCTTT
 GCTGCCGTGGCTGCTGGTTGGGACCAACTTGGTGGAGTGATAAGCACAA
 CAAGCATCACGCTTGAGTGACTCTGACTCTTGTGTTACTGCAAGTGTG
 GTTAAAGATTGAATCAATACCATCGTACTCATATCCTCAACATTCTTC
 AATCGCAACAGCCAACGAAATGGAGTTGATGAAGAGATTGCGACCGATC
 CATTCTCTTCTCTTATGTCGGGATCCAAGTACGGATTCTCCACTTCGT
 AAGATCCAACACTACATCTTCTACAGTCCCTCTCTCTCTCTCTCCCT
 CTGGCGCTGGACACCCCTAACGGTGCCTGAGACTCAAGTGAATCGAAAC
 GTCCCGATGCAAGAATTGTCGTTATCTCTGGCACATTACTTCTG
 TTGTGACCTCTTCCCAGCTCACGGTGTGGGCTGCTGCTTCCCTCTC
 TGGCCTCATGTCGACTATTGTTACTCCGACACATCAGTCGGAAAGAGT
 ATTTGAGGAGTATCAGCTGATTGGGIGACGGCTCAGTTGGAGAGCAG
 AGAAATGCTGTCAGGAAATCCATTCTCTGAGTGCTTGGGAGGAAT
 GCAATTACCGTGGAGCATCACTTGTCCCTCCATGCCAGGTAAGCAG
 CTIAATGTTGTATCTGTACCAATTGTTGACTCTGCTTCTGGCAACN
 CTGTTGGAAAGCTATGAGCCTAGCACATAATGGTGTATGCGACCATGA
 ACTCGATTTAAGGTCAAAATACCTTACTATGATCTCAGTCCGGTGCCTGA
 TGACGTGTGTC

Figure 7b

QPFVPPSSG WRKAHPAGPH WIDWYDGRDA TEVMDAFHTQ KGREMYKRLP ASAPETAAVL
 EASAAPYSQT ELNFRKLRDQ LESEGWWERD FVMEGKLLAI WASLVTGAAL TAESAPPLST
 FLLGLSMTNA GWLGHDIYHG VDKFSQVMRP FRAVAAGLGF TWNSDHNKH HALSESDSCC
 YCKCGLKIES IPSYSYPQHS FNRNSQRNGS

RLNQYHRTHI LNILSIATAN EMGVDEDIAT DPFPLPPVYVD PKYDPLRKI QHYIFYSPFS
 FLFALWRVDT LKVAVDSVES KREDAKNEWL YLLAHYFVLL TFFPAQWVVP AVFLSGLMSA
 LIVTPTHOSE EYFEEYQPDW VTAQFESTRN AVTINPSEW LWGGMQYQLE HHLYFSPMPR



GENERAL 2007 FORM

Figure 6a

TODESI = Thalassiosira pseudonana DESATURASE I

AAAAAAAAAAANNNNNGGAAAGCGAGATCAATCGAGCTGGTACCATGAG
 TTTCAAAAGTCACCTAACATTCACAGTGACAAAAGAGAGGGCCTCAG
 ACGTGCTGAGCAAAGCCTTCACAGGGAAATAGTAGGGGAAAACAGAA
 ATATTGGCAAATTATCTTACGTTCTGATTTATCTTCATTACTAAAG
 GGAAAACAATGCAGCTAAAAGCTACGTTGTACTCTTGAACACCAC
 CTCACCCCCGGCTTCGGTCCGGGTCGGCCCGCTTGCATCCTTCCTC
 CTCTCACAAATTATCATCCAACGAGCTGATAACGIGTCATTCACAGGGT
 CAACACAATAAAACATACTAATCAACCATGGAAAAGGAGGAGACGAGC
 CGCAGGCCACCAAGCGTAGTGGAGCATGAAATTGGCGAGAAGCCGAGA
 AGTACACCTGGCAGGAGGTGAAGAAGCACGTGAGTCTCCGCTTGTGTTGC
 TGCCGTTGGATGTCCTGCGTTGGATATGCAACGAGASTTCGT
 ATTGCAACTCAATTTCATGTCATCTGCAATCAACTCATCTGACCCAA
 CAACTCTGCCACCGTCCACCCATTCAAGATCACCCCCGACGATGCCCTGGG
 TAGTCCACCAAACAAAGCTACGACGTCTCAACTGGTACGACCAACCC
 CGTGCAGCCGTTGGATGTCCTGCGGAGACGACATGACGGACATCTT
 CGCCGCCCTCCACGCCAACGGCTCTCAGGCCATGATGAAGAAGTTTACA
 TTGGAGATTTGATTCCGGAGACTGTTGGAGCATAAAGGATCAAAGACAGTTG
 GATTGCAAGAAGGGATATGGTAACTTACGGCAAGCTTGTCAATGATGGG
 GATGTCAGTGGAGTAAGATGTAATGCAATCAAGTCTCGTTCAATA
 TGTGCAATGTTGGCTGGCGGTGGCATTGGTACTACTCGGACAGTTG
 GCAATGCAACATGGATCGGCTCTCTGTTGGGATTTGTTCTGGCAGCAGTG
 TGGATGGCTTGGCAGCAGCTTCTCACCACCAAGTCTTAAGCAACGAA
 AGTACGGAGATCTCGTTGGCATTGGGAGAATCTCATGCAGGGGTT
 TCGATGCACTGGTGGAGAACAGCACATGGCACCAGTGTCTCCAA
 CTTGCAACAATCTTCTTGGACAGTCAGGATGGTATCCCGATATTGATA
 CGATGCCACTCTTGCTTGGAGTCTCAAGCAGGCTCAGAGTTTCAGAGAG
 ATCAATAAGGAAAGGACAGTACCTCGTCAAGTACGCTATCAAATCCA
 GGCATTCACATACTTCCCATCCTCTCTGGCTCGCATCTTGGTTGA
 ATGAATCCTTCACAAACTGCACTGGACTCGGAGCTGCCCTGGAGAATGCC
 AAGTGGAGTGGAGAACGGCTGGACTTCAGTACCCACATTGGAGAAGCT
 TGGAAATCACCCCTCATTACACTTGGATGGTCTCTCTCCGGATTG
 GAAGGGTGGCTTCCATATTCCATCATGTAATTCTTCACTGCCACATGC
 TCCCTGGGACTTTCTCGCATTGGCTTTGGATTGGCACACAAACGGTAT
 GTCAGTGTACGATGCCACACCCGACCTGACTCTGGCAACTCCAAGTC
 CCACTACACGTAACATCATGGTGGACACGGCATTCCCCAATTCTTGTG
 GATTGGTTCTGGTGGATTGCAATACCAAGTGGATCACCAACCTCTCC
 CATGATGCCCTAGAAACAATATGCCGAATGCCACAAAGCTGTGGAGTCAT
 TCTGTAAGGAGTGGGTTGCAAGTACCATGAGGCGCGATGTGGGATGGT
 ACCGGTGGAAAGTGTGCAACATCTCCACGGTGGGATGGTATTTCTTGT
 GGAGATGGTGAAGGATTCCCTGCCATGTAACACCTATTACCGTCCGGC
 AGCTTCTCCGGTGGCTGGAGATGCAATGCAACTCATCGTAATAACTC
 ATTATAATGAAACAATGTTACCCCTGCACTGGAGGTTGCTTGTGTTGT
 CCCACCCCTCTTCTATTGTGATTGGTGTGATCATGAAACAGAGATAGTC
 TCTACATCAGATCTCTCCATTACGGTCAATGATTCACCAACAAACCATC
 ACATCAAACACTTGAATCTCCCTGTGGCAATCCCTCCATTGTACATT
 TACTCTCAAAGGTATATCTATTGGTCCCTTATTAAATTGGTGAATATTGA
 AGGGGAAGGATTCCATTCTTCCCTCTCTTCCCGATGATCCCTCTCACCT
 CTAATAACCTTCAACACACAACAAAGCAACAAACGGCAGATCAGACAAACA
 ACATGGCAGAAGCTATCCCTACCGTGCAACCGATCCAAAGGCGAAGAGCTA
 TTCCCTAGTCCATCTCAACGGCATGTCGGCTCCAGACCCCTATCCCTGAG
 AGTGAAGTTGTGATGTCGCTGATGTACTTTCCGCTTGTGATGTTCTGAGG
 TGTCAACACTCAGGGTCAACAAAGCAGCTTCGCTGATCGTAGTGGCGAG
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 CTTGAAAGTCGAGGGTACGAATAATTGGGGCGGAGN

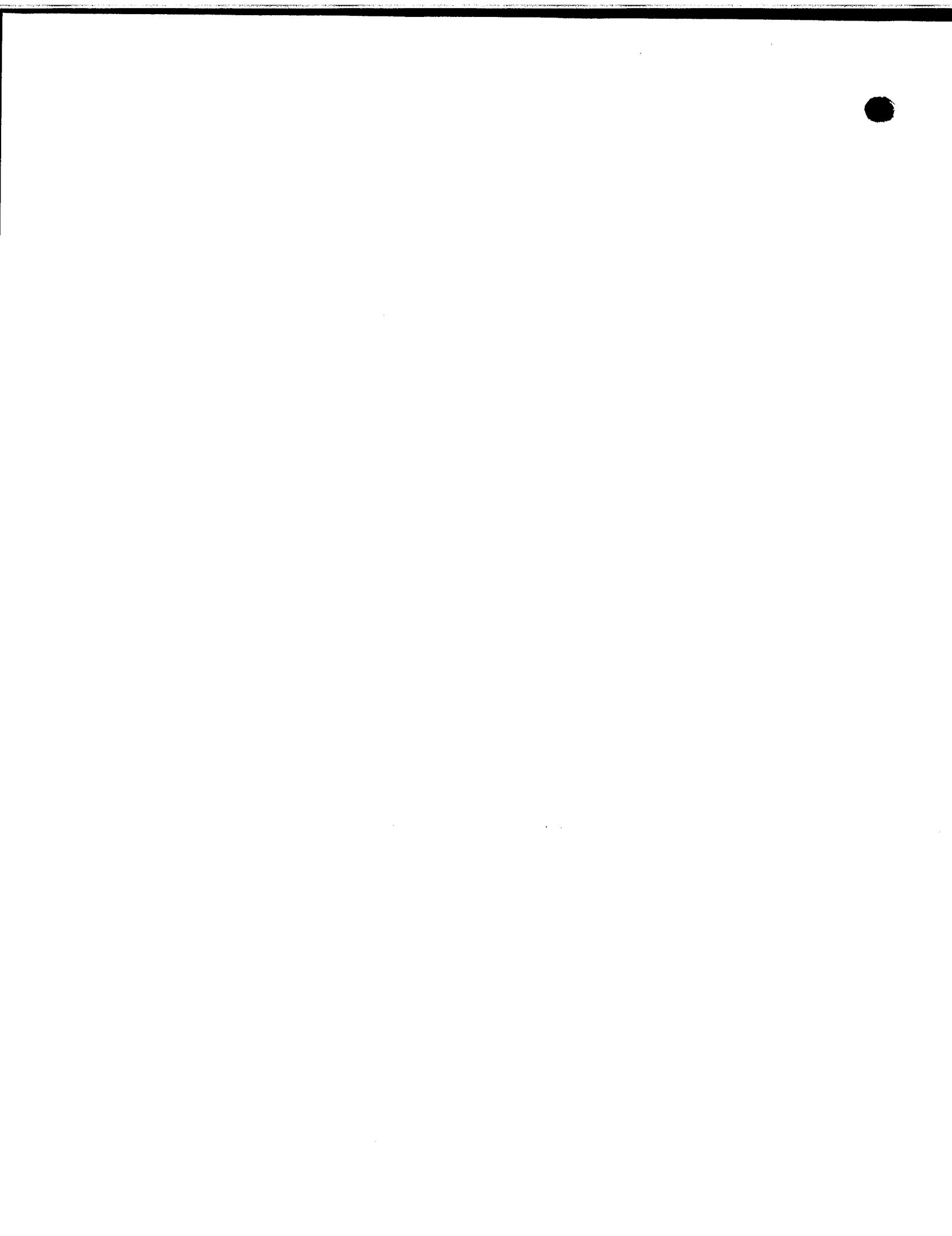


Figure 8b

GCT (Alanine) codon add by us to improve yeast expression

```

ATGCCCTCGAAAAGCAGGAGACCCACCCGGAGCTACCAAGCGTAGTGGAGCATTGAATTG
GCGGAGAAGCCGAGAGTACACTTGGCAGGAGCTGAGAAGCAAGATCACCCGGAGCAT
GCCTGGCTACTTCCACAAAACAAAGCTACGACGCTTCCAACTGCTACGACCCGGAGCT
GGAGGGCTGCTACCCAGACATGACGGACATCTCGCCGCCCTTCAC
GCTCAAGCTCTCGGCCATGATGAAAGTACTTTACATTGCAATTGATTCGGAGACT
GTCGAGGATAAGGATGAAAGAACCTGGATTCCGAGAGGGATATCGTGATTTACGGGCC
AAGCTGTGATGATGGGATGTTCACTCGAGTAAGATGTTATGCTACAACTGCTCG
TTCAATATGTCATGTTGGGTTGGGCGGGCCATGGTACTCTGGACAGTTGGCA
ATGCACTTGGGATGGGCTCTCTGTTGGATGTTCTGCGAGCTGTGGATGEGCTTCG
CACGACTTCTTACCCACCAAGTCTTACGAAACGAGTACGGAGATCTCGTTGGCTTC
TTTGGGAGATCTGATGCGAGGGGTTCTGATGCACTGGAGAAGCACAGCACATGGC
GACCGATGCTTCCAACTTGCACAACTCTTCTGCTGTCAGGATGTTGTCAGGATGTTGTC
ATTGATACCATGCTCCAACTTGCACAACTCTTCTGCTGTCAGGATGCTGTCAGGATGTTGTC
ATTAAGGGAAASGACAGTACCTTCTGCAAGTACCTTCTAAATTGCAAGGAACTCACATAC
TTTCCCCTCCCTCTGGCTGCCCTCTCTTGGATGAAATGCTTCAAAACTGCTTC
GGACTCGGAGCTGCTGGAGAATGCGAAGTGGAGAAGCTGGACTTCTGTC
GCACTTTGCAAGAAGTGGAACTTCACTACGTTGATGTTCTCTCTCTG
GGATTGGAAAGTGTCTTCCATATTCATCATGTTTCTGCACTGCGCACATGCTCG
TCCGAACTTTTCTGCACTTGGCTTTGGATGGGACACACGGTATGTCAGTGTACCAT
GGCACACCCGACCTGACTTCTGCAACTCCAAAGTCACCACTACAGTAACATCATGTTG
GCAACGGGATTCCCCAAATCTTGTGGATTGGTCTGGGGTTGCAATACCGAGTG
GATGACGACCTCTTCCCTGATGATGCTGAAACATATGCGAAAGTGGCCACAAGCTTGTG
GACTCAATTCTGTAAGGAGTGGGGTGTGAGTACCATGAGGTGATATGTGGGATGGTACCG
GTGGAAGTGTGCAACATCTCTCCAAGGTGTCGGATGATTCTCTGAGATGGTGAAG
GATTTCCCTGCCATGTA

```

Figure 8c

Amino acid sequence deduced from the cDNA sequence

```

MAKKGDDARA ATKRSGALKL AEKPQKTYWQ EVKIGHITDD AAVVYQNNKVY DVSNWYDHEP
GAVVFTTHAGD DMTDIAAFFH AQQSOAUMKK FYIQLIPEF VEHMDQRQLD FEKGYRDRLA
KLVMMEMFKS SKMYYAYKCS ENMCMWLVAY AMVYTSDSLW NHIGSALLLG LFWQQCGWLA
HDFLFLRQVKP QRKYQDLYGI FWGDLMQGFS MQWWKNNHNG HHAPEWLNNS SLDSDQGDFD
IDTMELLAWS LKQAOQSFREI NKKGKUSTVVK WALKFQAFTY FPIILLARIS WLNESEFKTAF
GLGAASHNAK LELEKRGLOV PLLEKLGTLW HYTWMLVLSF GFGRWSLPYS IMYFTTATCS
SGLFLALIVFG LGHNGMSVYD ATTFREDIWLQ QVTTTTRNITG CHGIPQFFVD WFCGGLQYQV
DHHLFPMMPK NNTIAKCHKLV BSFCKENGVK YHEADWPGT VEVLOHLSKV SDDFLVEMVK
DFPAM

```



CHLOROPHYLL A B6 W/100K

Figure 9a

TPESK = Thalassiosira pseudonana DESATURASE K

TATGTCCACCCCCCCCCCTGGTTGTCCACCTCTGTCTTCGATCTGGGACC
 CGGGTCTCGAGTTGCCAGACCTCTCAAGCGGGCCCATAGTAGACGGACTT
 GATCTGTTTGTGATACCTGACGTCACCGATTTCGCGGCTAACGCCA
 CTTTCGTAACCTCCACCAAGGTACGACTGACTTGTGCCCGTAGATATCTCT
 GATAACCTCTATGCCAAAGCCGATCAAATCGAATTGATTGTACTGTAGCAA
 GGATAAGCAGATGGATAAGCGGGGATCTTCATGTCGACAAAGAGGAAGAG
 AGAGAGTATGTGTCGGCGAGGGTGGATAGGTGAGAGAGACGGGATGAC
 AGATTGTACATTATCTTCCCTCCAAACACTTAAACAGGACCTCAGTCTG
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 AAGTCCGAGCTCGCCCAACACACACAGCCCCAAATCAGCATGGTGTGCCGT
 CCAACTCCACTCCGCCACCGACCCATCCACTCCAACAACAAACACCC
 CACACCTAGTCTCGACATTACCGACTTTGCGTCCGCCATCCAGGGGCA
 GACCTCATCCTCTCGCTTCCGCAAAAGACGCGCTCGGTGCTGTTGAAAC
 ATACCATCCACGTGGAGTTCCGACGCTCTCATTCAAAAGCTCCAGATTG
 GAGTGATGGAGGGAGGGCGTTCCGGATTGGTTTACAGTTGGACTGAT
 TCTGACTTTTATACTGTGTTGAAGAGGGGTTGGAGCGGGTTGGAGGA
 GAGGGGGTTGGCGAGGAGGGGATCGAAAGAGATTGGATCAAGGCTTGT
 TCTTGTGTTGAGTTTGGTACTGTTGTACAAGATGTACTACGTGCG
 GATATTGATCAGTACGGTATTGCCATTGCCATTCTATTCTATTGGAATGGGAAC
 CTTTGGGCATTCACTCGCACGTGATTCAACCGATGGAAATCACCGTG
 CATTGCTCAGAACAAAGTTACTCAACAAAGTTGGCTGGGAGCTCAGCACATGCC
 ATGATGGGTGGAGTGCCTTACGTGGGAGCTCAGCACATGCC
 TCATCCATATAAGGAATGTGTTGGATGGGGTGGAGGGAGGGAGGGAGGA
 GGGGGGAGGATTTGCTTTGGAAGAAAAGGATCAGGTGAGACGGAGATGAC
 AGAGAGAGAGAGACTTATCGTGTGAAGTCTGAGATGCAATGTC
 TGAGCGACACAACCTCAACGCAATTCCATTCCACTTCAACTCGCC
 GAATCAGACACGACTTATCCATGGTATCATAAATACCAACACCTCA
 CCTCCATACACCTCATGGTATCATTTGAACTTCCACAGGATTGGAA
 CCTCTTGTGATTGACACTTGGCAAAGTATTCCAAACAGGATTGGAA
 GTTGGCACATCCGGACGATTATATCATATTGATGCCAATGACGGTATGG
 TTGGTATGGAAATGTCATGAGTTTGGCTATGAGGTCAATTACGATGG
 GATATAATGATGGGATTACCAATCTACTTTCATGGAGTACTGAGGGGAGTT
 GGATTGTTGTTATTGGCATTGGCGTGTGGAGAGTTGTTGGCGACCGAT
 GTTATTGTGAAATCACGTCAATTGAGGGTGTGAGTTATGGAACGAGGATT
 TGTTGGTGGTGGAGTCATGTAGATGAGAAGAAGATTGTCAAGCCAACG
 ACTGTATTGGAGATAACCCAATGGAAAAGACTCGCGAGGGAGGCATTGAA
 AAGCAACAGCAATAACAAACAAGAAGAAGGAGAGAAGAAACTCGSTACCAT
 CGGTTCCATTCAACGACTGGGAGCTCAATGCCAGACCTCCGTGAAT
 TGGTCTCCAGGCTCATGGTTCTGGAAATCACTTTCTGGGGGACTCTCTCA
 TCAGATTGAGCATCACTGTCTCCAGCATTTGTCAACAAACTACTGTC
 ATATCCAGGATGTTGAGAGACTACGTGTCAGTACGGAGTTCCGTAT
 CAGAGTGGAGACTAATTGTTGTTGCTTATGAAAGATGATTAGTCATT
 GAAGTTTTGGCTAAAGCCAAGTGTGAGTAGGTGTTAGGTTATTGAGAGGT
 GTCCAGTTGTCATTCATTAAAGCGTGAAGTCAATTGCTCTGGAA
 ACAAGGTTGTCATACCACTCTCTTGTATTGATTCGCTGCGTCACACAT
 CTCCGTGAGGAGTTGACCTCACTATTCTAATTGGAAATGTCCTTTG
 CGCTGGTGAAGTGGAGCAATACACTCCGNCAGAAGAGAGACTGCA
 ATGCAAGAGGAAAGAGGATATACTGTATGAGTCCGAAAGAATCGATGACGCG
 CGGTGAGGTGGTGTACATCACTTGTGAGGACCAACGTGGAACCGCATGTC
 TGAAGAGGTCCATACCTAAACATTGAGGGCTTGGCGACCAAACCTTAG
 GAGAGATTGAAATGTCATGGCTATTGTTGTTCTGTGCGGAAACCTTGATA
 AGGAACAGCAACCAACACACCGGGC



Figure 9b

REF ID: A9A86076

MCINGNLAST AQLKSTSKPQ QQHEHRTISK SELAQHNTPK SAWCAVHSTP ATDPSHSNNK
QH AHLVLDIT DFASRHPGGD LILLASGKDA SVLFETYHPR GVPTS LIQKL QIGVM BEEBAF
RDSFYSWIDS DFYTVLKRRL VERLEERGLA RRGSK EIWIK ALFLLVGFYV CUYKMYTTSD
IDQYGTIAIAY SIGMGTFAAF IGT CIOHDGN HGAF AQNKLL NKL AGWTLDM IGAS AFTWEL
QHMLGHHPYT NVLDGVVEER KERGEDVALE EKDQVRDDDR ERESLFV

QESD PDVFSSFPLM RMHPLHTTSW
YHRYQHLYAP PLFALMTLAK VFQODFEVAT SCR LYHIDAN VRYGSVWNVM REWAMKVITM
GYMMGLPIYF HGVLRGVGLF VIGHLACGEL LATMFIVNHV IEGVSYGTID LVGCASHVDE
KKTVKPTTTL GDTPMEKIRE EALKSNSNNN KKKGEKNSVP SVPFNDWA AV QCQTSVNWSP
GSWPNHFSG GLSHQIEBHL FFSICHTNYC HIQDVVESTC AEYGVPYQSE SNLFVAYGKX
ISHLKFLGKA KCE



0094865 17 Feb 04 05:13

Figure 10aTpDES = *Thalassiosira pseudonana* DESATURASE N

ATGGACTTTCTCCGGGGATCCT
 TTCCGGACACTCGTCCTTGCAAGCAGTTGTCATCGGATTTGCTGCGGC
 GTGGCAATGCTCTACCGCCGAGCATCGTGGCAAGCCCTGTCACATTAA
 GCAATGGTAAACTCAAAATCCAGAATCCATGGCAAATTGTAACGACCTCTCA
 TCGTTCAGCATCCAGGGGGGGCTCTTCTTGTCAAGGTGCG
 CGACGGAACAGCTCTATTGAGTCACACCATCCCTCATACCTCGAAAGA
 ATCTACCTTCAGATCCCTCAAGTACGGAGGTTCCGTGACTGAAGACTCT
 GTTTCCTCATGCCACCCCTAGACGAACTCAATGGTGAATCTCCGTACGA
 TTGGAAAGGACATTGAAAATGATGATTGATCTGACCTACGGAGCTCG
 TAATTGAGCACTTTCTCCTCTCGCCAAGGAAAGGGGAGTTCACTCGTT
 GAGTCGTCGAAGGCAACACCTCAGCGGTGGATGGGGTTCTACTGCTCCT
 TGGCGTCGTTCTCCTCAGCATCCCAATTATATTGAGTGGGTCGTGGACTT
 TCGTTGTCGTCACTCCCATCCTCGCTTGGCTGGCGGTTGTCATTACTGG
 CACCGATGCTACTCACTTGCATTGAGCAGCAACTGGATTGAAATGCTGC
 GCTCCCCATATCTCCTCCCTCTCCTATCGAGTCGGCAATGTTGGTATCATC
 ATCACGGTCAATTGGACATCACGCATACACCAACATTCCAAAAGAGATCCA
 GATCTTGCTCACGCTCCACAACCTCATGAGAGAACACAAGAGTATCAAATG
 GAGACCATCTCACTTAAATCAAACACAGCTTCGCGGGATTCTCTTCACTCT
 GGTGCAATTGCACTGGGTTGAAACTTAACGAAACGACGGTGAAGAGCA
 CTAACCAAGCTTCATACTAACACGTTGTTGCGGTGGAGAACAGATGTCATC
 GTCGGCAACATTACTCCATTCTTGGACGTTGTCACATCTTGTGA
 CTACACTTTGCCCTTTTGGCGTTCCGGTGTGGAAAGGCCATCGTTGG
 GCGACTGTACCGAATGCCATACTGAGTTGTCATTGTCATGCTGAATACCGCA
 AATCAAATCACCTCATCACACCGTGTGACATGCTCCGATAACAACTTTT
 ACAAGGCATCAAGTTGTAACGCTCAGAACCTTGGCGATCAAGTGCCTTT
 TGCTTCATCTCTCGGGAGGTCTCAACTACCAAAATTGAAACATCATTGTT
 GCGGACGGTGAACCAATTGCCATTGCAAGCTTGGCCCCGGGTGTAGAGC
 GTTTGTGTGAAACACGGGGTGAACATACAACTCTGTTGAAGGATAACAGA
 GAGGCCATATTGCAACACTTGCACATACCAAAAGATATGTCGACCAAGCC
 TACTGATTGA



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Figure 11a TpDES0 = Thalassiosira pseudonana DESATURASE_O

ANNTCTCCCACCCNGCCAGGCTCTTTCAGGTCCACGGAGATAACACACTTC
 TTCCCACCAACTTCTGCTCCATACCATCGGAAGAAAAGAGGAGATTATC
 TTGACTTCTGACGGAGGAGTGGGATGAAAAGAACTTGAGTGCTGGTAAGGG
 CTGATTTCTGAGAAGGAGAAGTCAGCTGGAACGAAGTTCATGGAGTTT
 TGTGGCAACCCATTGAGACGTTGCTTGGTGGAGGAAGGTAGCGAGGTTG
 AGCATGCAAACAGAATGGTATAAATCACTAAGATGTCACTCCAAATGACA
 AGTAGGAATAGCAATGACCGAGATGGTGTACAGATGTTAGAGATGGAGAGA
 TTAAGCGAATGGCTGGATGATTAGGATATGCAATGCAAATGAAAATGTTAGAT
 TCTTGCTAATAGACTTTGAGACAACGTCCTCTGCAGAAAAGGACAATA
 CTAATTAATATAAAACCGACTCGGAGAGAACATGACATGGCAAGTTGTCA
 CTATGGAATTCACTACGTCGTTGACAGGAAGTCACCGTGGCTCGGCGA
 AGAACACAAACAAAACCGAGGCTCACATTCTACTCTGTACAGTTCATAG
 TCAACACCACCAATACGGATGCCCCCACAACGCGCATATCTCCCGCATCCGC
 AACCGCATTCCCAACACGGTACCGTTGGCTCTGGCGACAACAAACGGA
 CCCCCGCACCCAAATCGTCCGAACCCCTAAATCTCTCAAGGGCAACGAGG
 TCGTCATCAACGGCACAATTATGACATTGCTGACTTTGTCCATCTGGGA
 GGAGAGGTGTCAAGTTCTTGGTGGGAATGATGTTACTTATTCAGTATAA
 TATGATTCTACGGTATCATACGGGAAACATCTGGAGAAGATGAAGGCTG
 TTGGAAAGGTTGTAGATTGGCAGTCCGAGTGGTGAATGGTGACACCG
 TTGACGTTGTGTGTCAATTCTCTTGGCATTGATATCCAACCTGA
 CCTCTACACACCTCTTGGTACCATAGCTACAGTTGACACACCCCTTG
 AACCGAGAGATCAAATCAGAAGTGTCAAGATGTCAGTGTACGTGGGGGTGAG
 TTGGGACAACAGGCTACTTCTCCGTGCCCTTTCTACATCGCTCTCTT
 CTTCAACCATGCAATACTTCTGCCCCACATGCAACACCTTCAACCACCTAG
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 CAGGCATTCACTGGGTTGAAATGTCAGGACGGATGCCAATCACGGAGCTGC
 CAGTAAGCGTCCCTGGGTCATGACTTTGGGATTTGGAAACGGGATTGGA
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 GTTGCATTCAATGACTATCCCATTGGACACCCGAAGAGAAAGTGGTGGC
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 GTACGGTGGAAATTCAAATGGAGAATGATTCAATTGTCAAGAGGGAGAAGT
 ACGGCGTTGCAATTGAGGATGATGTCATTACTTGAACATTGTCAAGCCCC
 TTGATGAACATTGGTTGAGCTGGTCTACCTTGGAAATCATCATGTGAT
 GGGAAATCAGCGAGAGTCTCACTCTCAGTGTCTTCTGTTGTCACACA
 ACTTCATCAATTGGATCGTGAATCTTACGGCTGACTTCAAAAAGACCCGA
 GAACAAAGTGTGCTGGTCAAGTCGCAAGGTGGAGACTTCGCTCACCTATGG
 GGCTTIAATTCCGGATGTCATTACGGGAGGACTCAACTTCAAGGTGGGAC
 ATCATCTCTTCCCCGTATGAGCAGTGCTTGGTATCTTACATTGCAACCT
 ACGGTTCTGAGGTTTGCAGAAGAACGGACGGGGTGAACCTACGCTTATTATCC
 TTGGATTGGCAGAATTGGTATCAACATTCAAATACATGCACTGGCTCTG
 GTAGTGGAGCCAACGGGAGCTCAAGCCGTTGCTGGAAAGTGCCCTAAAGT
 TTAGTTGACTGATTGTCGGAGGTGCTGCTGGTCTCAACTAATGTTAG
 GAGTGATGTTAAAGCCTTCTTGTGTTTGTGTTGCTTCTGTTATTCACTGA
 TATCAGTTTCGATATGTTGCAATTGTAACCTCCACTTGCACACTCAAAC
 AAATCTAGCATAACATTCTCATCCGAGTGTCAATTGAAACGACTCATT
 ACGCAATGCGCTCTCTCATAAACCCGAACAAACTCGACCGACTTCATACTC
 TAATCGTCCATTCTGGCAGCTGCAATTCCAGGCTCTAGCAGCAGCTCTCTT
 ACTCAACTCCATGGACTCAACTTGCATCTGCCCCCGCATCAATCTCAT
 GCAACCGTGCCCCCTCTCATACCAAATCTGCTTGAACATOCAGTAATCATAG
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 TTGTTATTGTGATATACTGTGCTGGTGGCGCATGATGCTCCGNTGZN
 GTTGGNGACTGTGCAFTGGATGCTGCTGAAACAGTCGGTGCAGTGTAGG
 GGAGGGTGTGTTCTGAACTGAGGAGATGCCGAAACTGATAGGGGGTG
 GTGCAGCGCTATAAATTGGCAGGGCGAGCGCGAAGGAGAAGCCACAAGGCCAATACAA
 CAGAAAGTTAAATGAAGGACGTAATTCTACACAGTCCAGTGGCGAAGT
 TACAAAC



Figure 11b

GCT (Alanine) codon add by us to improve yeast expression

ATGGCTCCCCCAACGCCATATCTCCGCAACCGCATCCCCACCAAAACAGGT
 ACCGGTGCCTCTGCCGACAACAACGACCCGCCACCCAAATCCGTCCGAAACCCCTCAAATCT
 CTCAGGGCAACGAGGTGTCATCAACGGCACAATTATGACATTGCTGACTTTGTCAT
 CCTGGAGGAGAGGTTGTCAGGTTCTTGGTGGAAATGATGTTACTATTCAGTATAATATG
 ATTGATCCGATATCATACGGGAAACATCTGGAGAAGATGCAAGGCTGTTGCAACGGTGTGTA
 GATGGCAGTGGACTACAAGTTCGACACCCCCCTTGAACGAGAGATCAAATCAGAAGTG
 TTCAAGATGCTACGTCGGGGCGTGAGTTGGCACAACAGGCTACTTCCGTGCCCTT
 TTCTACATGGCTCTTCTCACCAGCAATACACTTCGCCACATGCACCACTTCACC
 ACCTACGATCAGTCGGTACAGAGTGGTGTATTTCATGCAATTGTTGGTATTTCACAG
 GCATTCACTGGGTTGAATGTCAGCAGCATGCAATCACGGAGCTGCCAGTAAGCGTCCC
 TGGGTGAATGACTTGGGATTGGAAACGGATTGGATCTAACAAATGGAATTGG
 ATGGCACAGCATTGGACTCATCAGCCTAACACTAACATAGTGAGAAGGATCCCCGATAGC
 TTCAGCTGGAACCTATGTTGCAATTCAATGACTATCCCATTGGCACACCCGAAGAGAAAAG
 TGGTGGCATAGGTTCCAGGGAGGCTACTTCCTCTCATGCTGGACTTACTGGCTCCCC
 ACTGTTAACCGCAATTCAATTGATCTTCGTCAACGTGGGCTCAGTACGTCCGAATT
 CAAATGGAGAATGATTTCATTGTCAGAGGAGGAAGTACGCCGTTGATTGAGGATGATG
 TACATTTCATTGCAACATTGTCAGCCCTTCATGAAACATTGGTTGAGCTGGTCTACCTT
 GGAATCATCATGTTGATGGAATCAGCGAGAGTCTCACTCTCAGTGTGCTTCTCGTTG
 TCTCACAACCTCATCAATTGGATCGTGTACCTACGGCTGACTTCACAAAGACCGGAGAA
 CAAGTGTGCTGTTCAAGTCGAGGGAGACTTCGTCACCTATGGGGTTTATTTC
 GGATGCTTACGGGAGGACTCAACTTTCAGGTTGAAACATCATCTCTTCCCCGATGAGC
 AGTGCTGGTATCCTTACATTGCACTTACGGTGTGAGGTTTGCAAGAAGCACGGGATG
 AGCTACGCTTATTATCCTTGGATTGGCAGAATTGGTATCAACATTCAAATACATGCAT
 CGCGCTGGTAGTGGAGGCAACTGGGAGCTCAAGCCGTTGCTGGAGTGCCCTAA

Figure 11c Amino acid sequence deduced from cDNA sequence

MAPPNADISR IRNRLPTKIG TVASADNNDP ATQSVRILKS LRGNEVVING TIIYDIADFVH
 PGGEVVKFFC GNDVTIQQNM IHPVHTGKHL BKKKAVGKUV DWQSDYKFD PPEREIKSHV
 FKIIVRGREF GTTGYFLPAPF EYTALEFFTMQ VTFATTCCTPFT TYDHWYQSGV FIAIVFGISQ
 AFIGLNUQHD ANHGAASKRP WNNDLLGPFT DLIQSNKRNW MACHWTHHAY TNHSEKDEPS
 FSSEPMFAFN DYPICHPKPK KWHRFQGGYF LFLMLGLYWL PTVENPQFDIY RQEGAQYVGI
 QMENDFTIVKR RKYAVALRMW YIVLNIVSPF MNNGLSTWF GIIIMLGTSF SLTLSVLFSL
 BHMFINSORD PTADPKKIGE QVCWFMSQVIE TSSTYGGFIS CCILWGLNFQ VEHHLFPRMS
 SAWYPYIAPT VREVCKKHM STAYYPWIGQ NLVUSTFICMH RAGSGANWEL KPLSGSA



Figure 12A

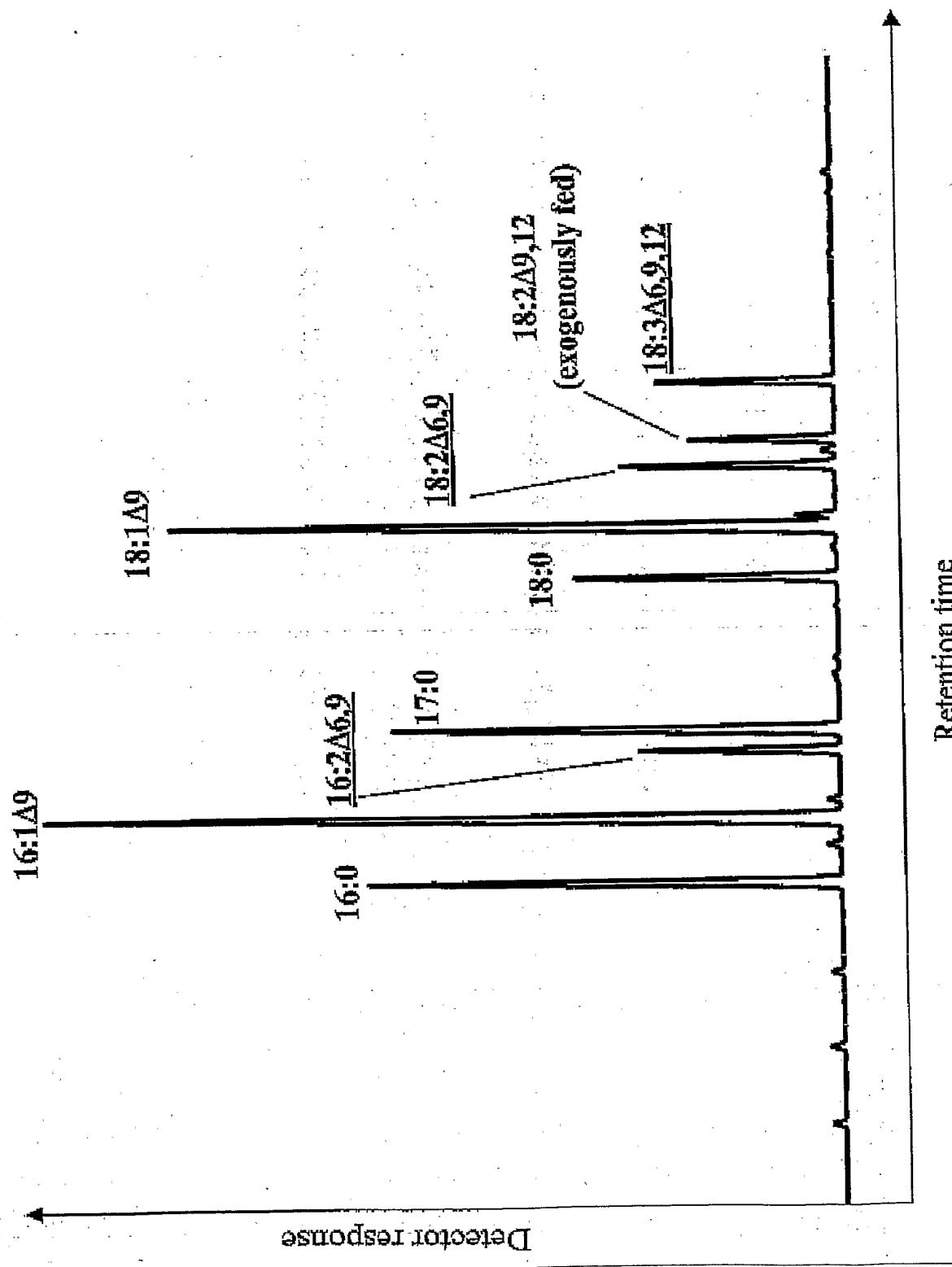




Figure 12B

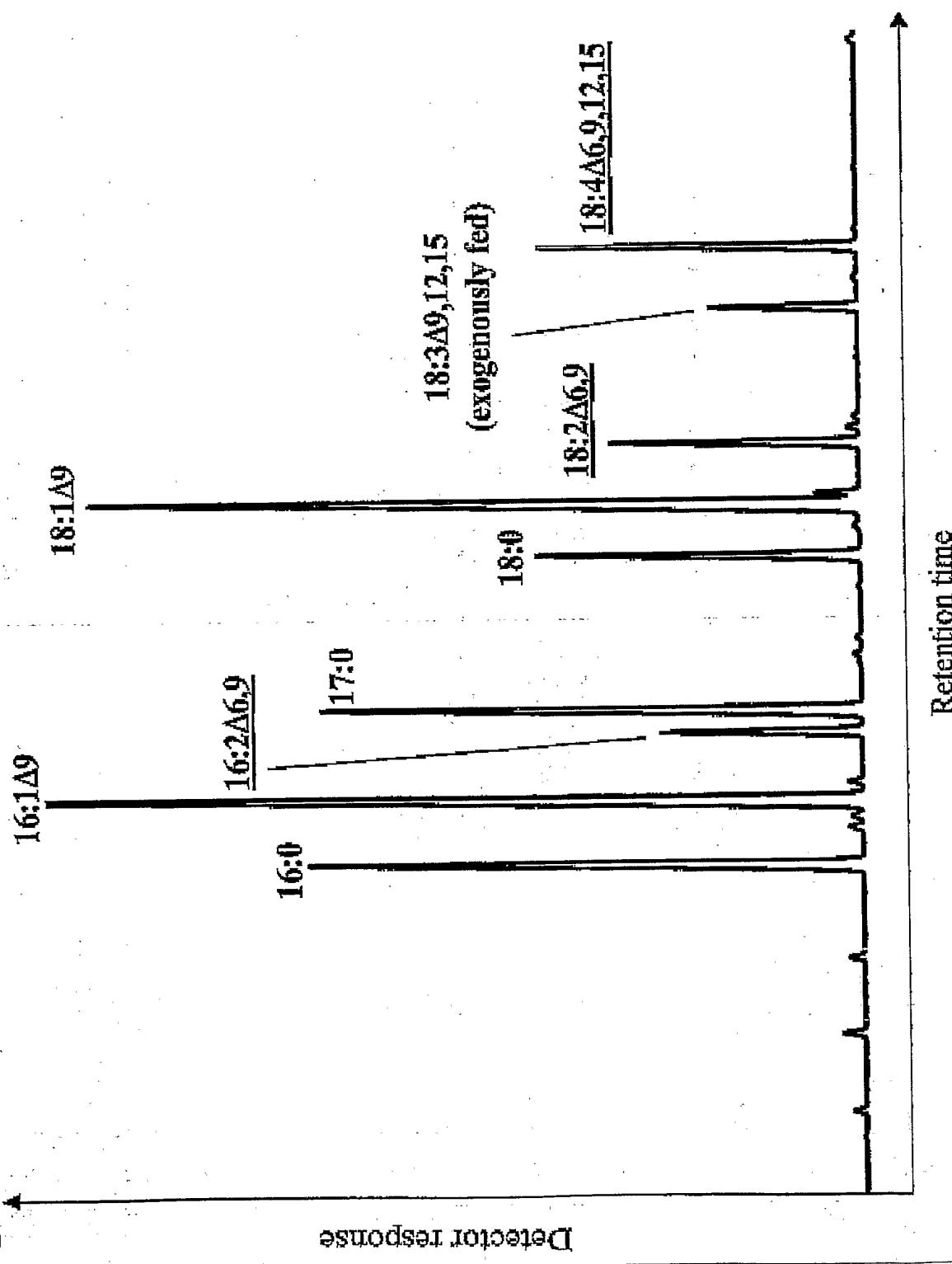




Figure 13

